

Figure 1: Examples of Nuclease Stable Ribozyme Motifs

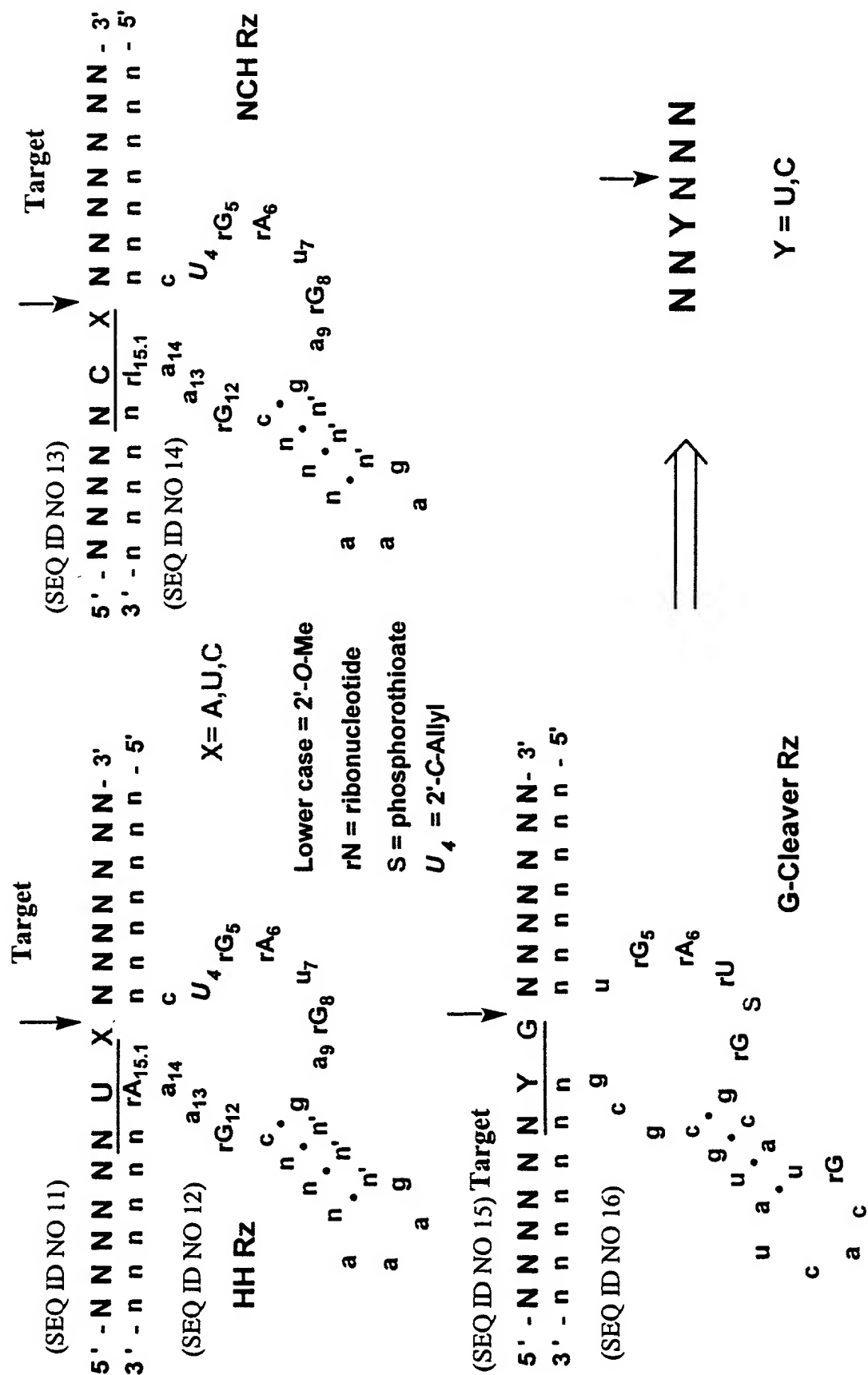


Figure 2: 2'-O-Me substituted Amberzyme Enzymatic Nucleic Acid Motif

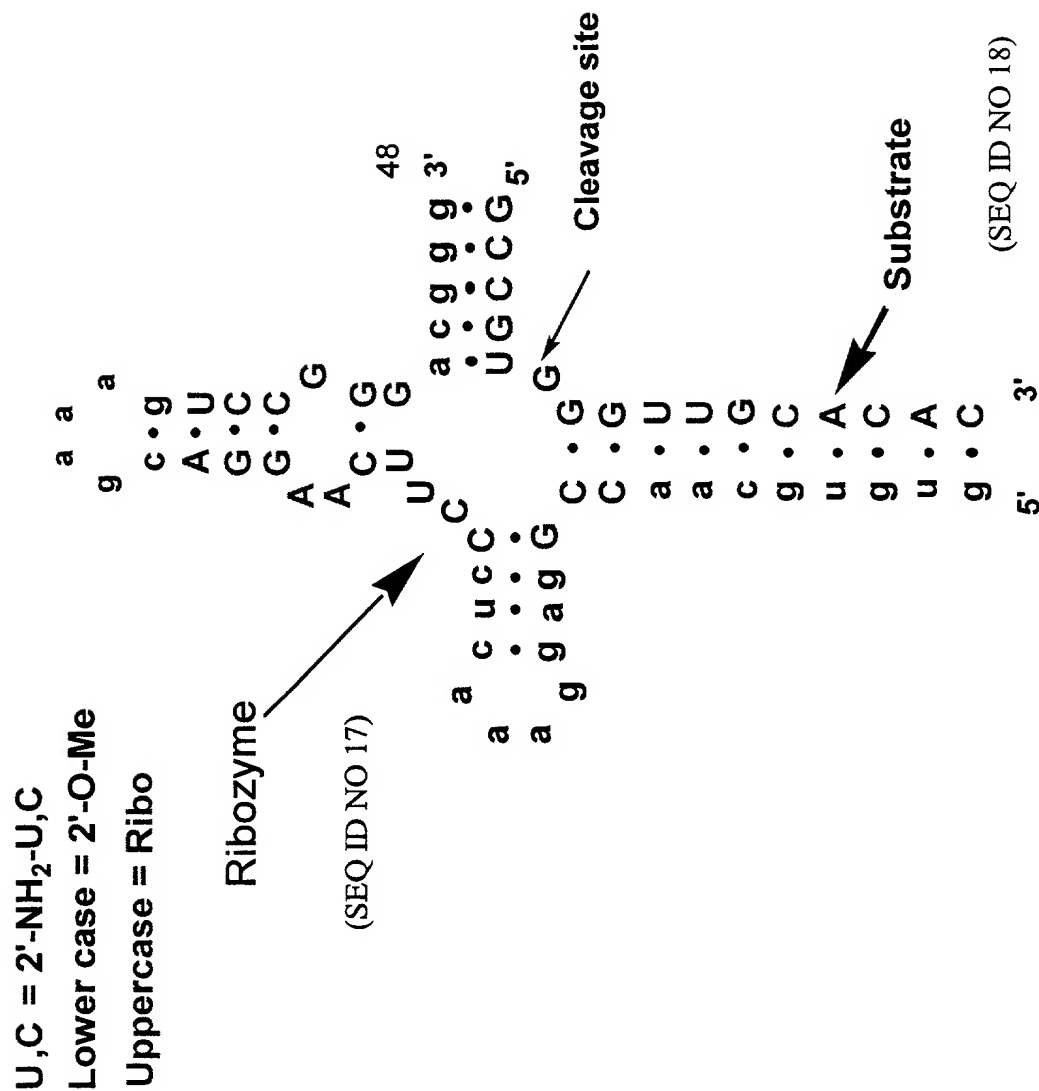
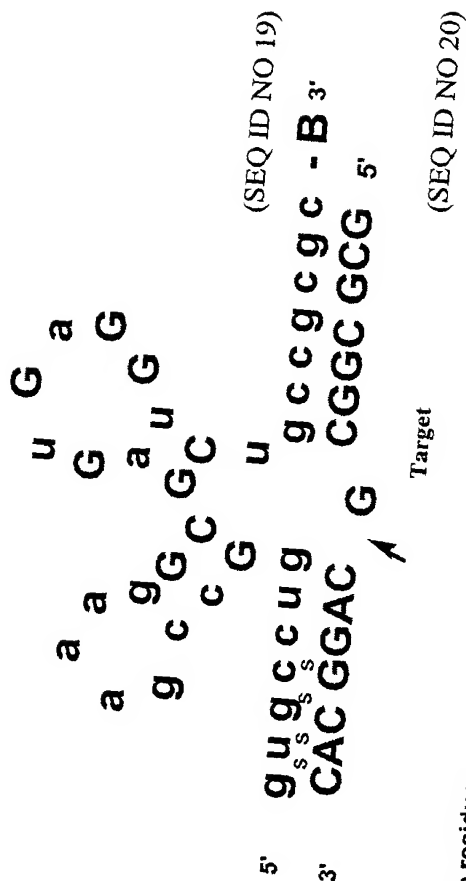


Figure 3: Stabilized Zinzyme Ribozyme Motif

Zinzyme A-motif RZ



Legend

Uppercase indicates natural ribo residues

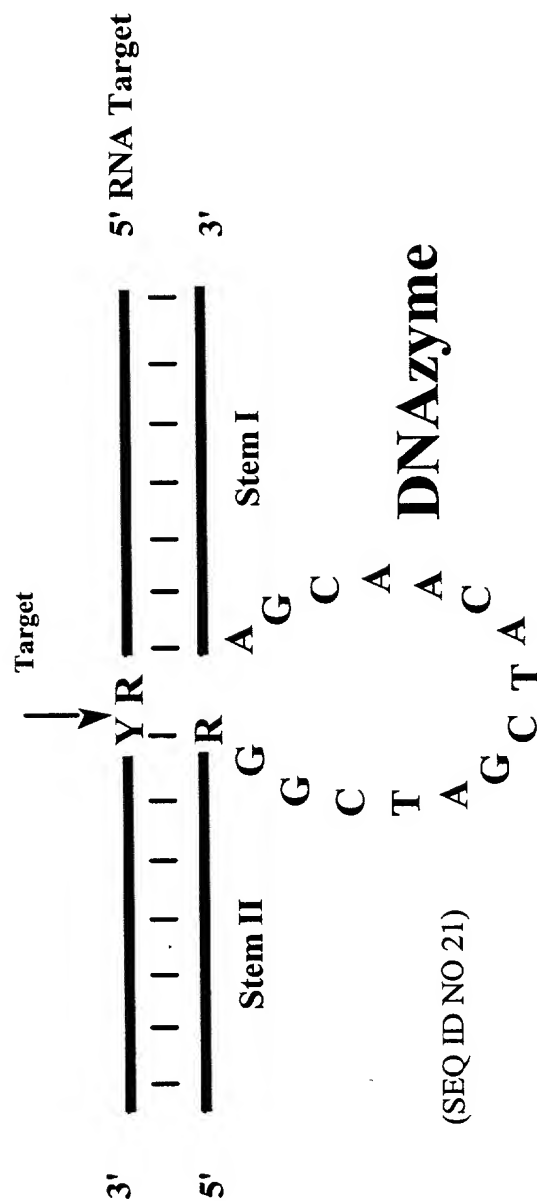
C indicates 2' - d-NH₂-C

Lowercase: 2'-O- Me

Subscript _s indicates phosphothioate linkage

B: 3'-3' abasic moiety

Figure 4: DNAzyme Motif



Legend

Y = U or C
R = A or G

Figure 5. Detection of Target Sequence Using a Cis-Blocking Sequence

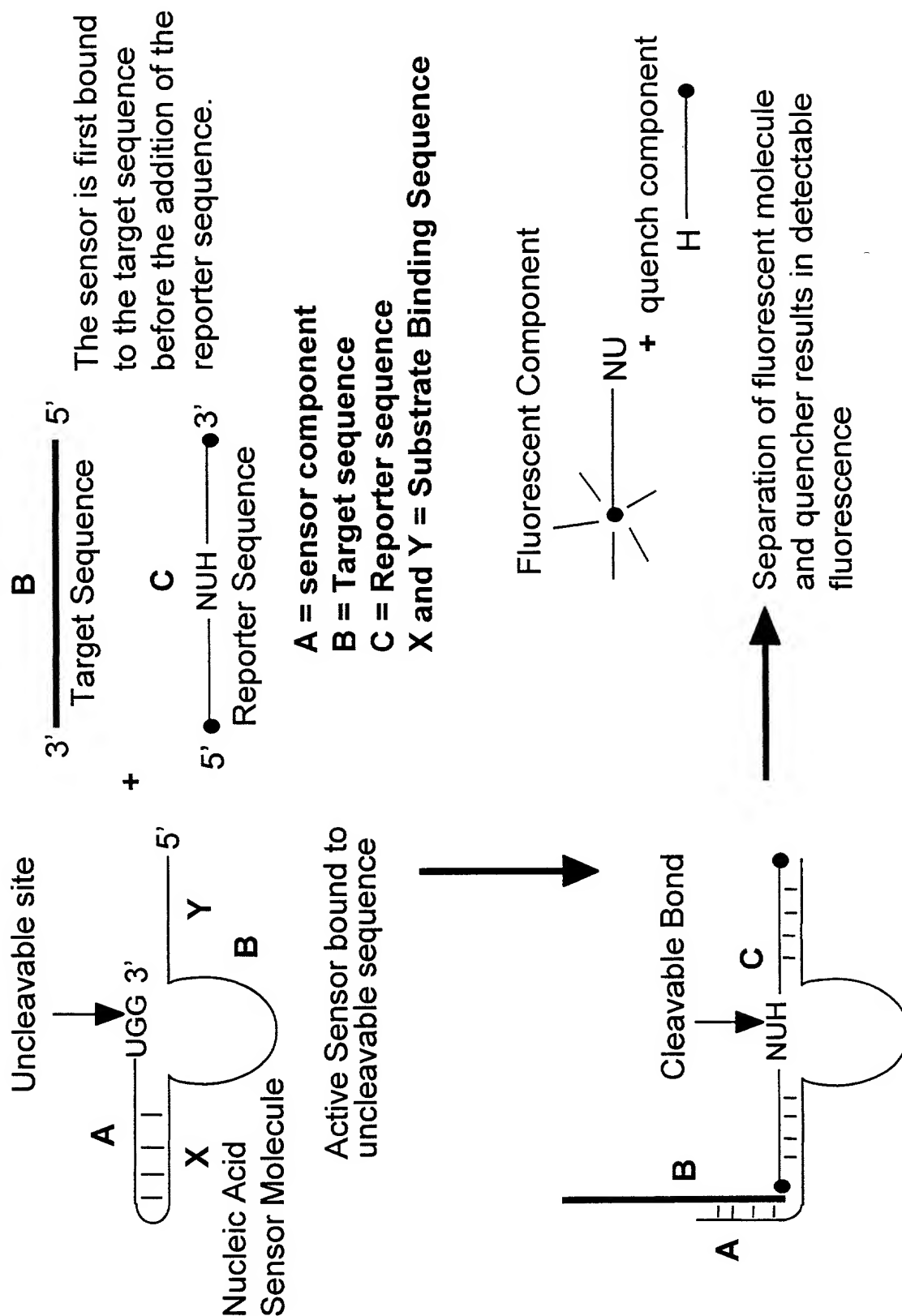


Figure 6. Schematic Diagram Representing the Two Primary Configurations of the Diagnostic effector molecule

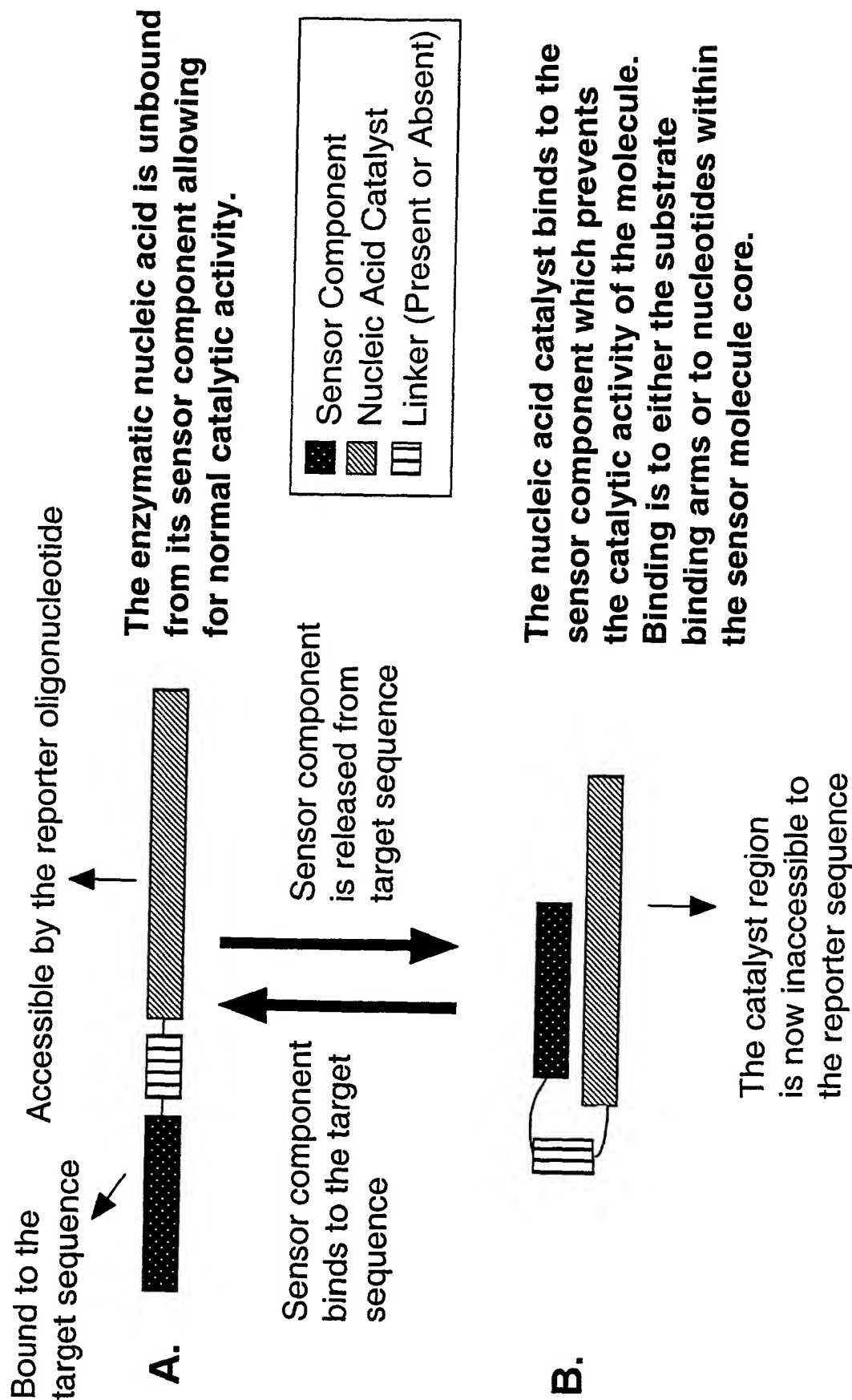


Figure 7a. Examples of Diagnostic Effector Molecules

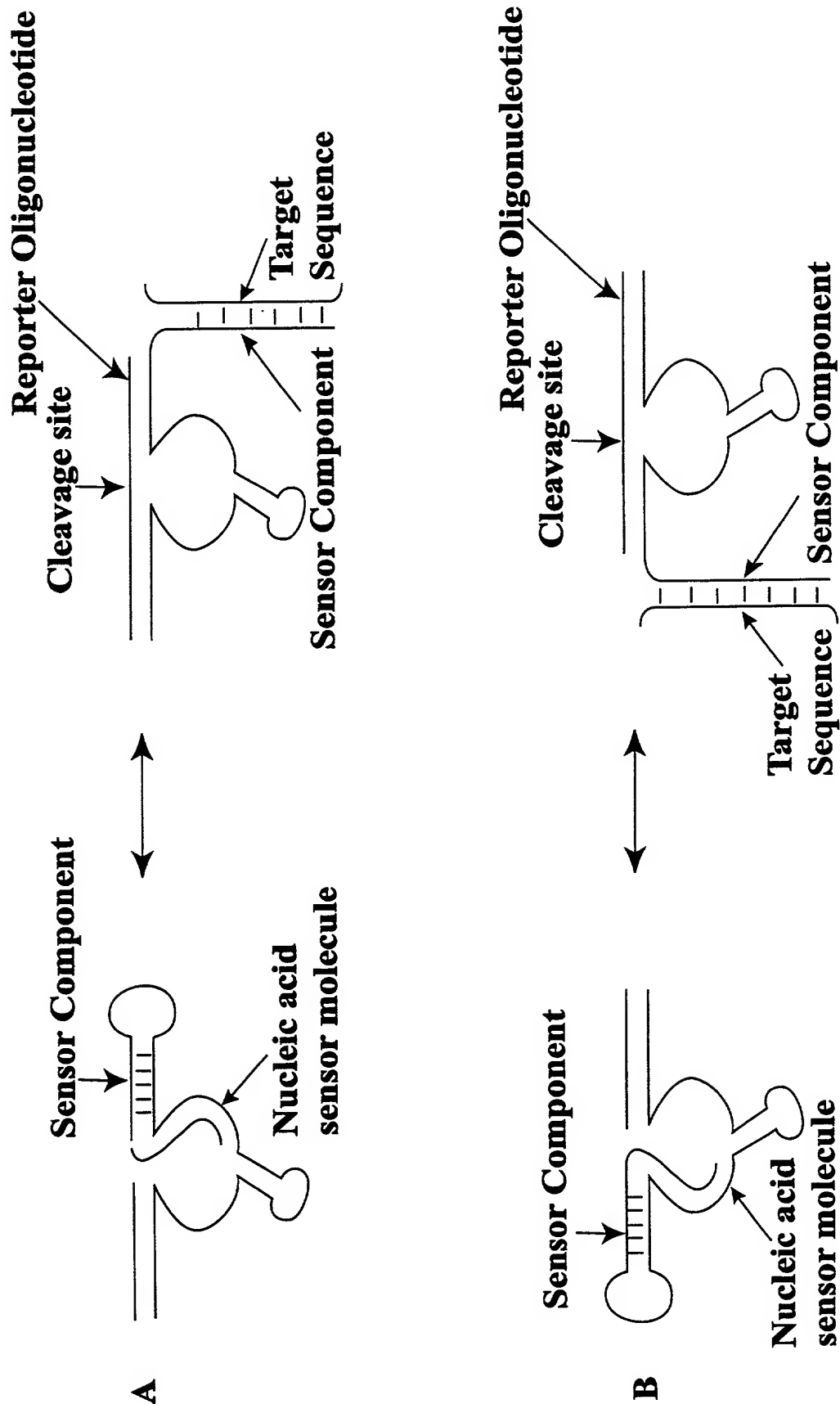


Figure 7b. Examples of Diagnostic Effector Molecules

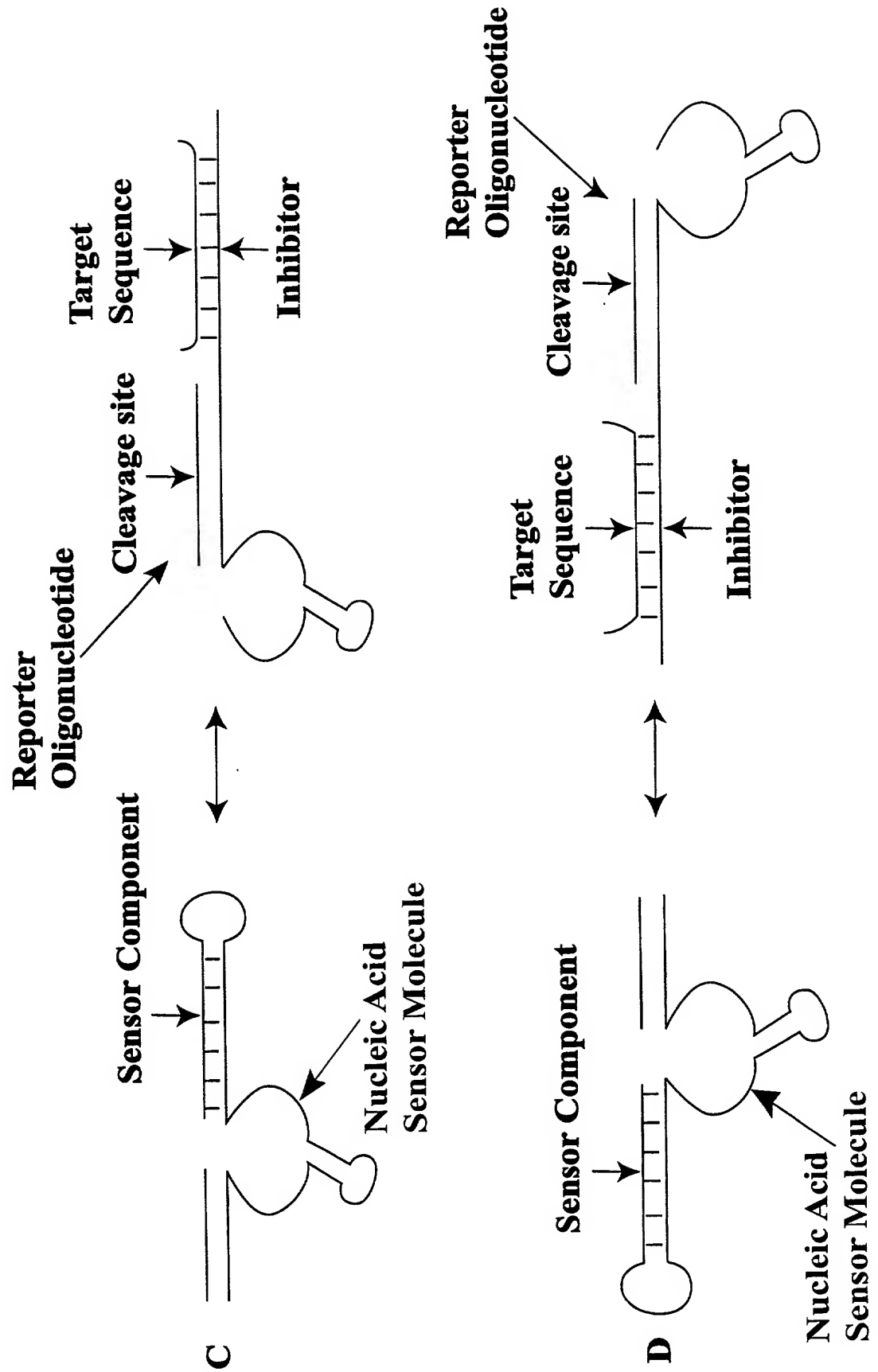


Figure 8a. Examples of Diagnostic Effector Molecules

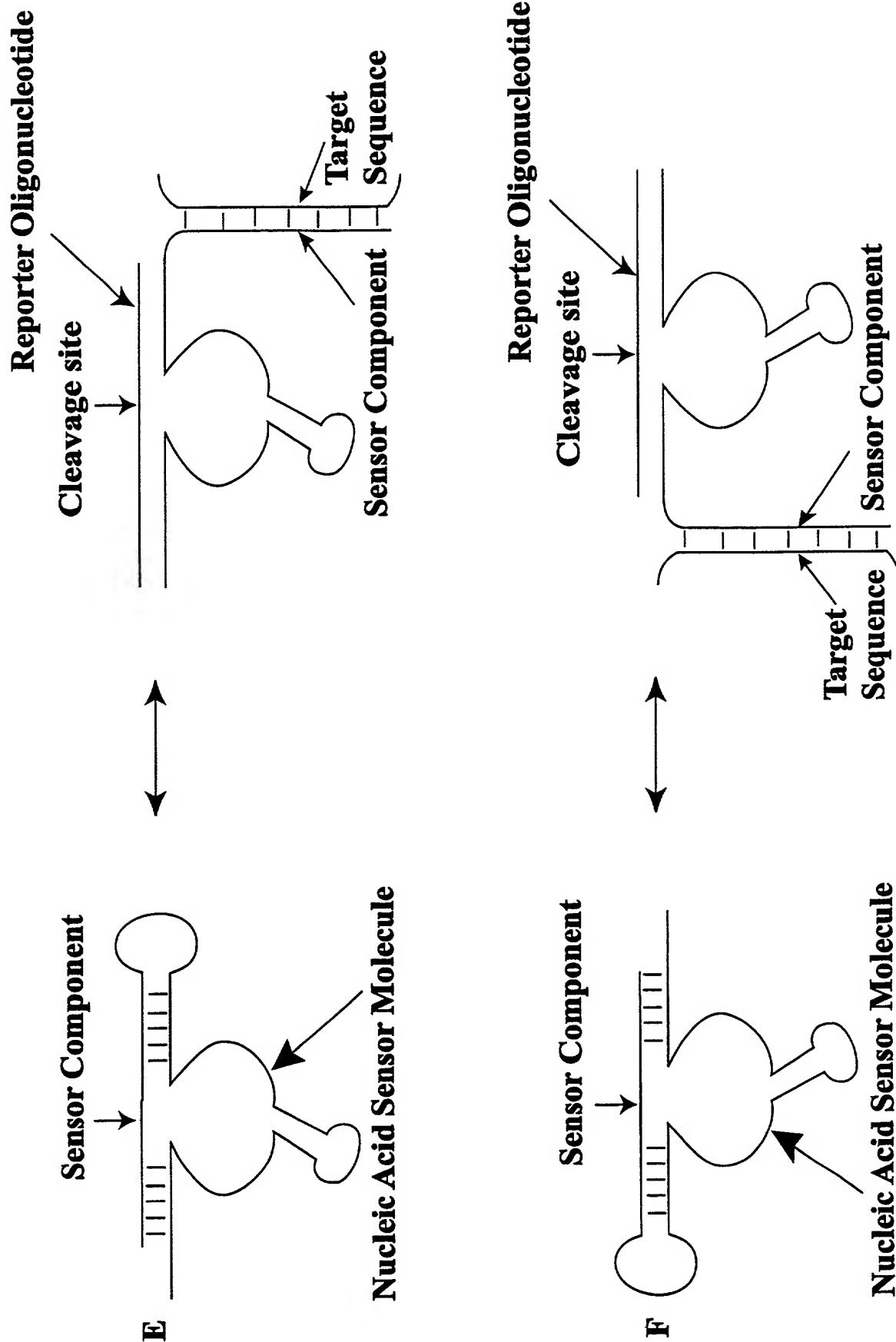


Figure 8b. Examples of Diagnostic Effector Molecules

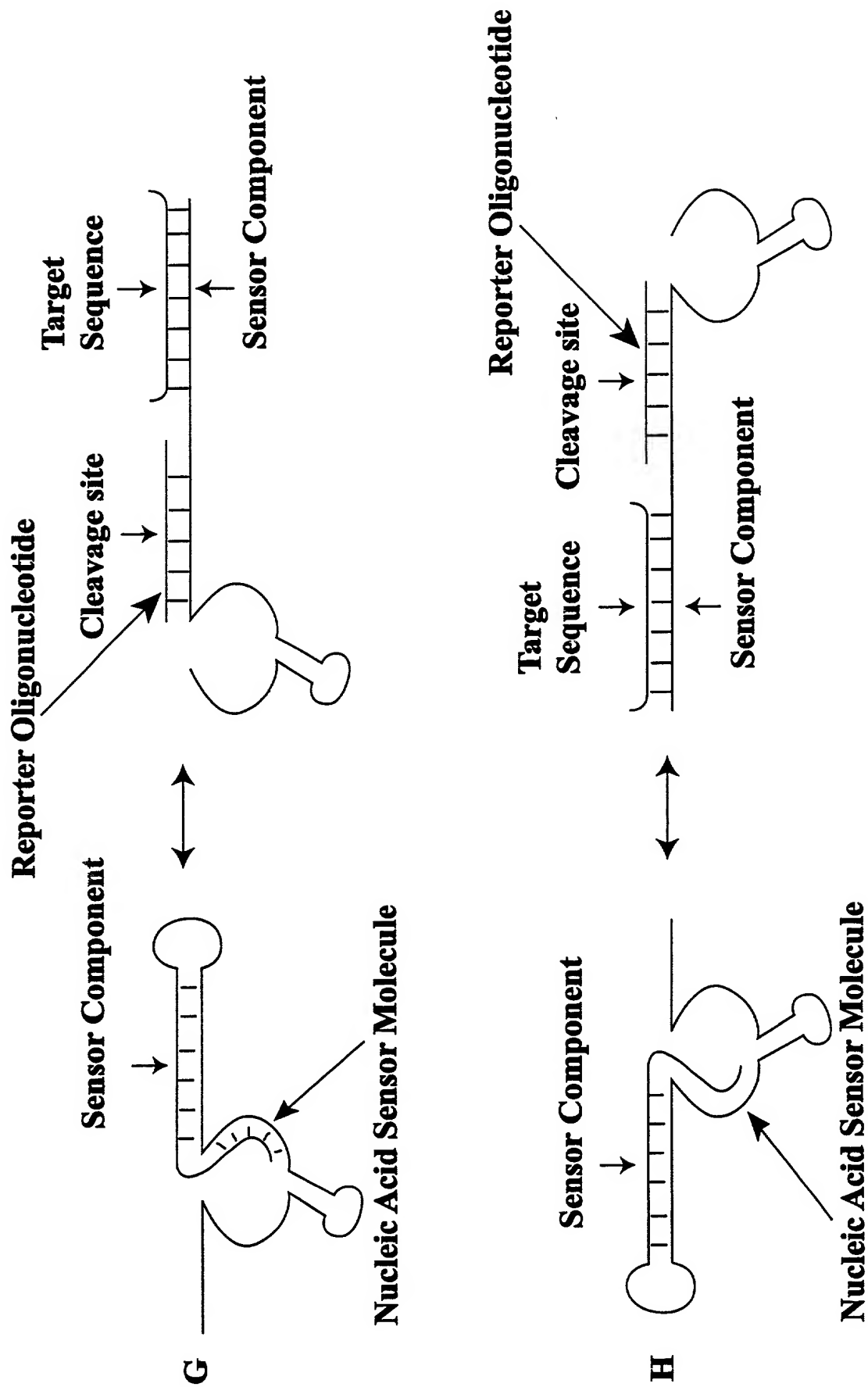


Figure 9. Examples of Diagnostic Effector Molecules

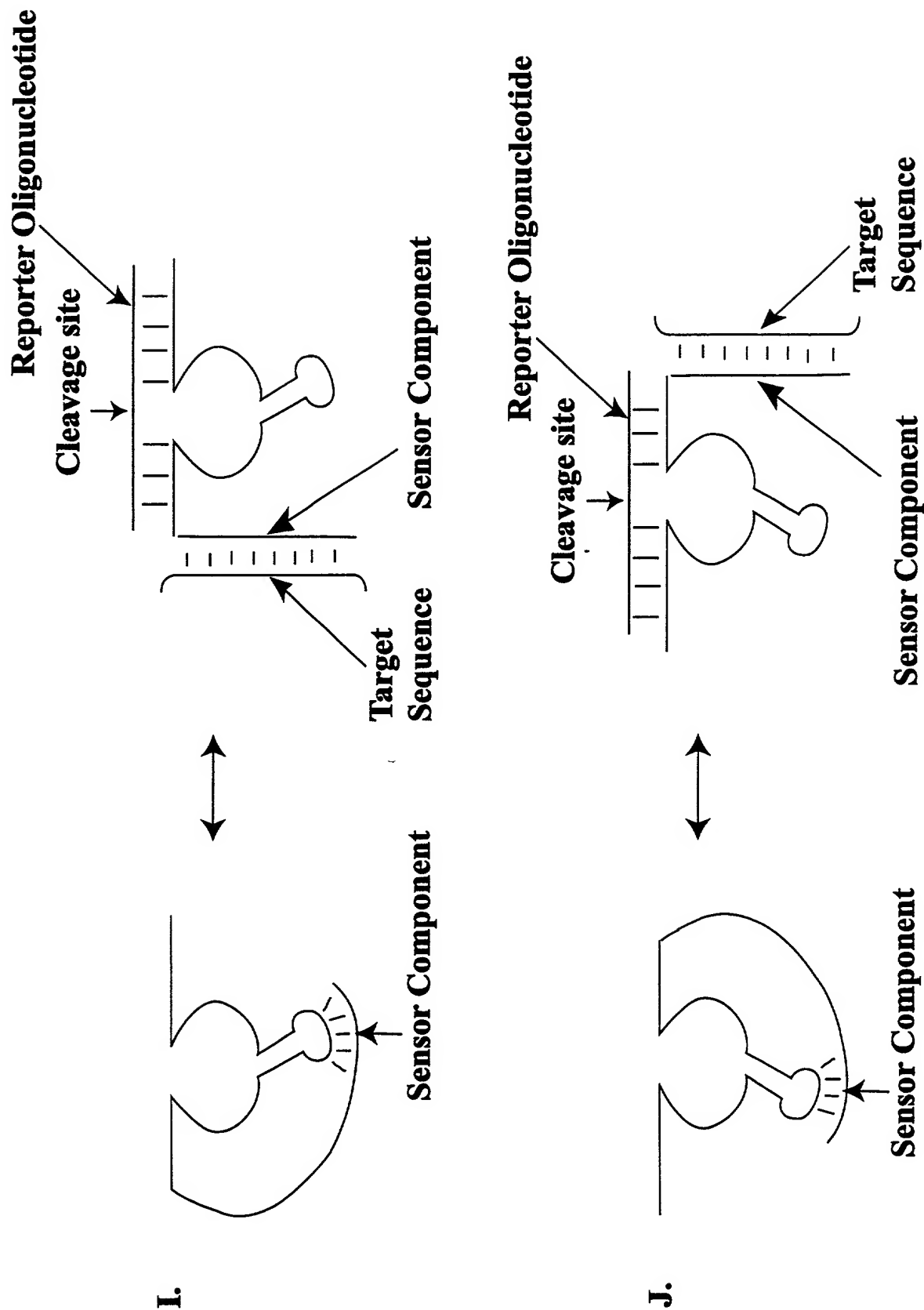


Figure 10: Examples of Diagnostic Effector Molecules

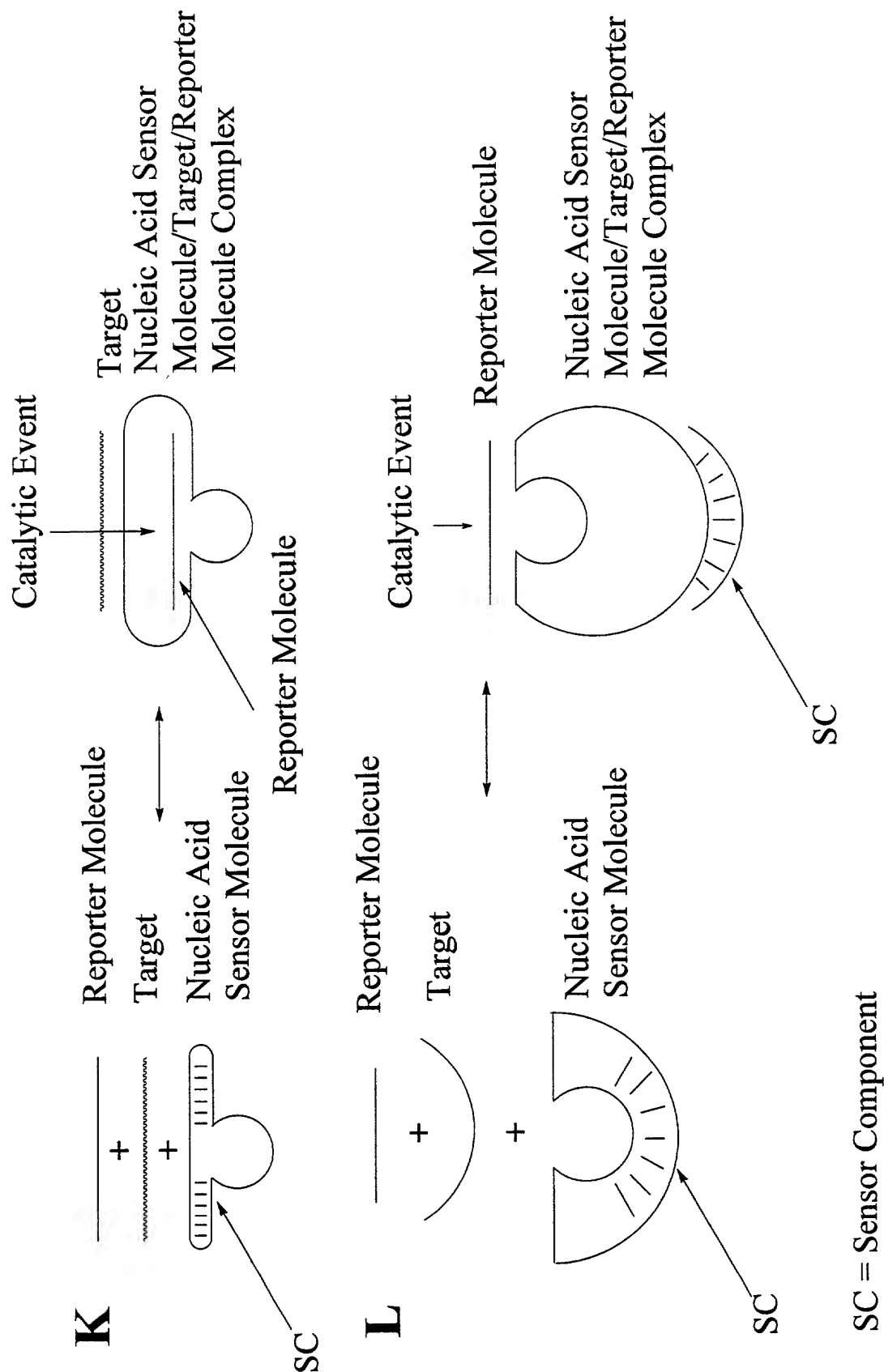


Figure 11: Examples of Diagnostic Effector Molecules

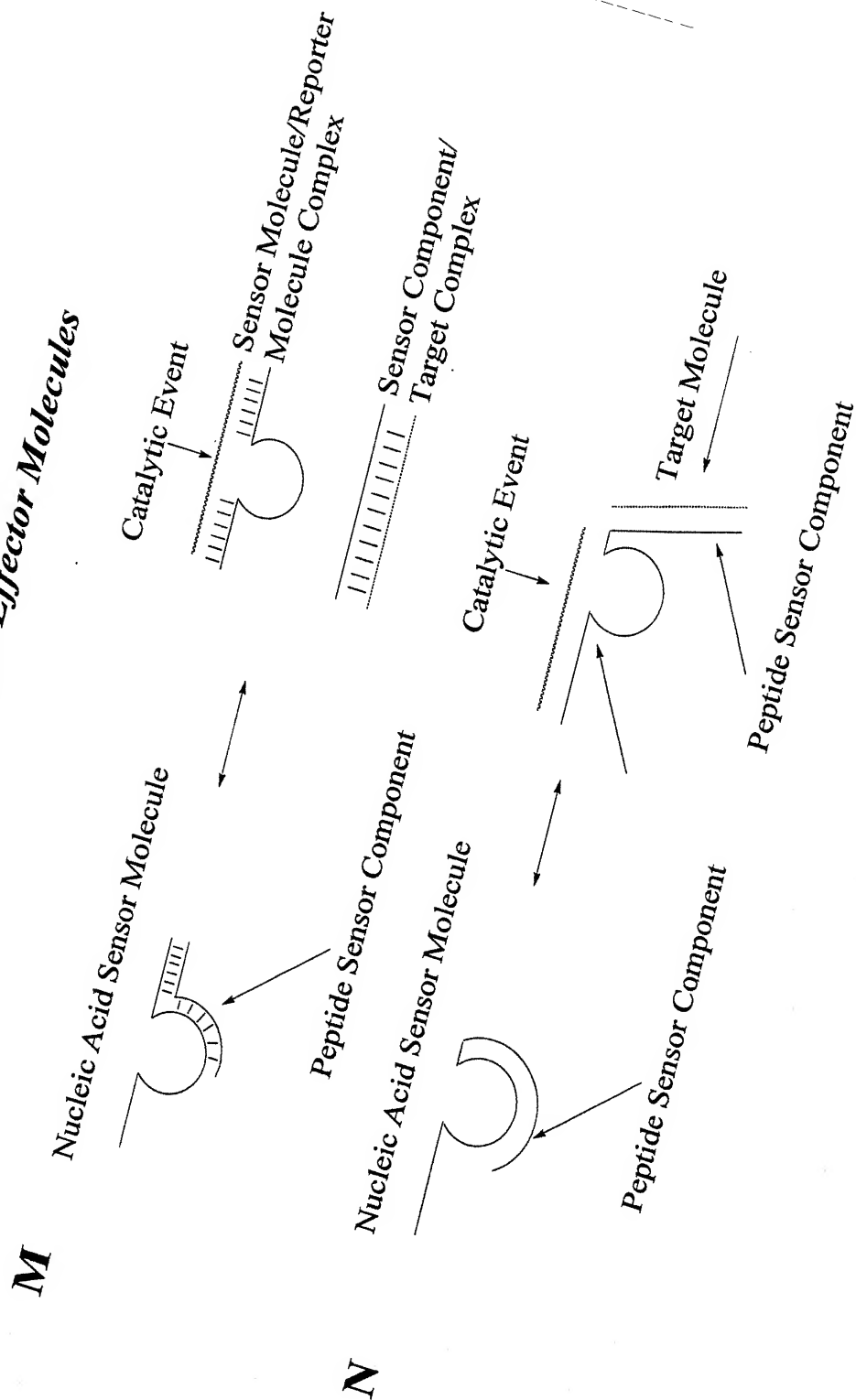


Figure 12: Examples of Diagnostic Effector Molecules

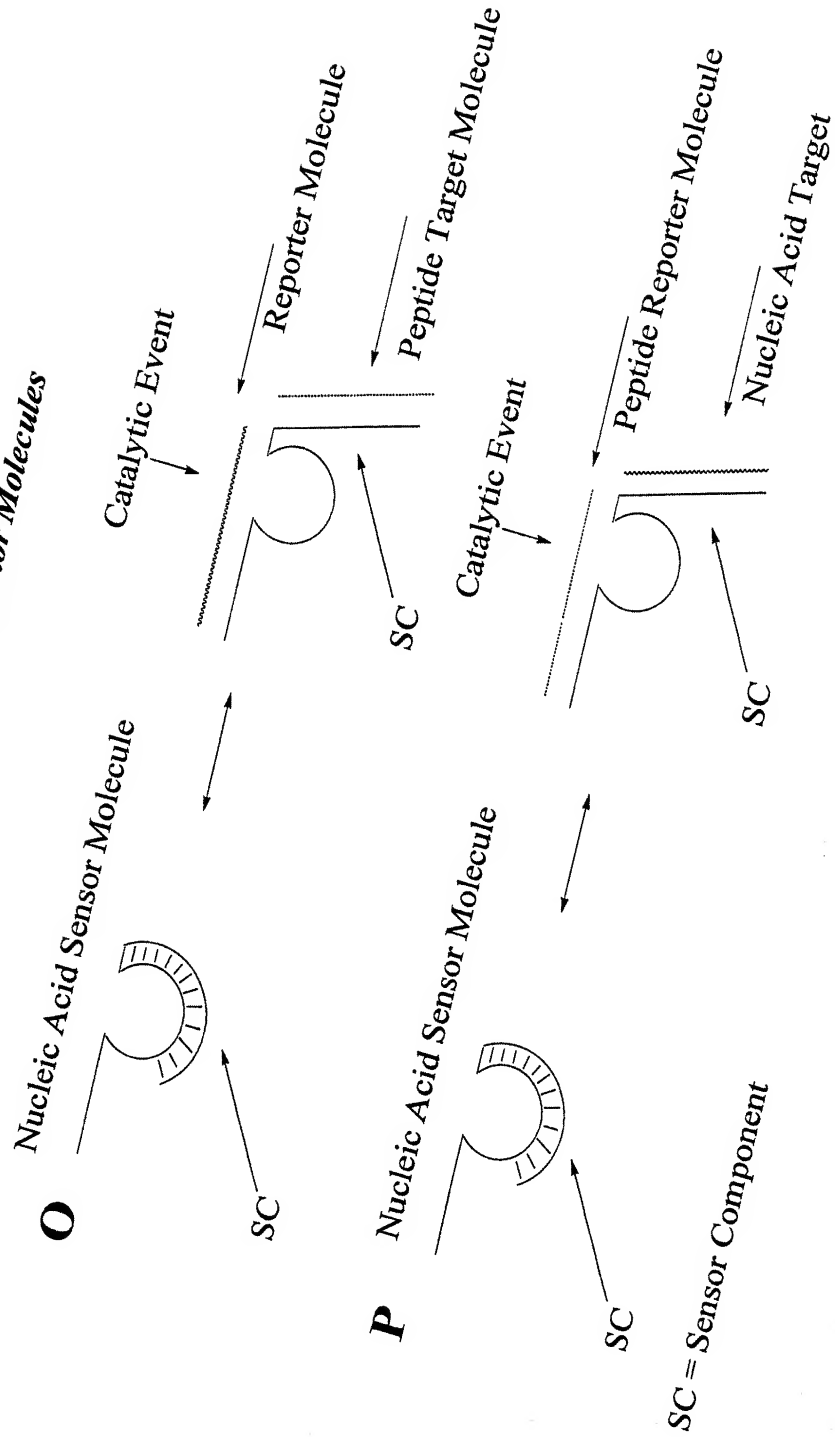


Figure 13: Examples of Diagnostic Effector Molecules

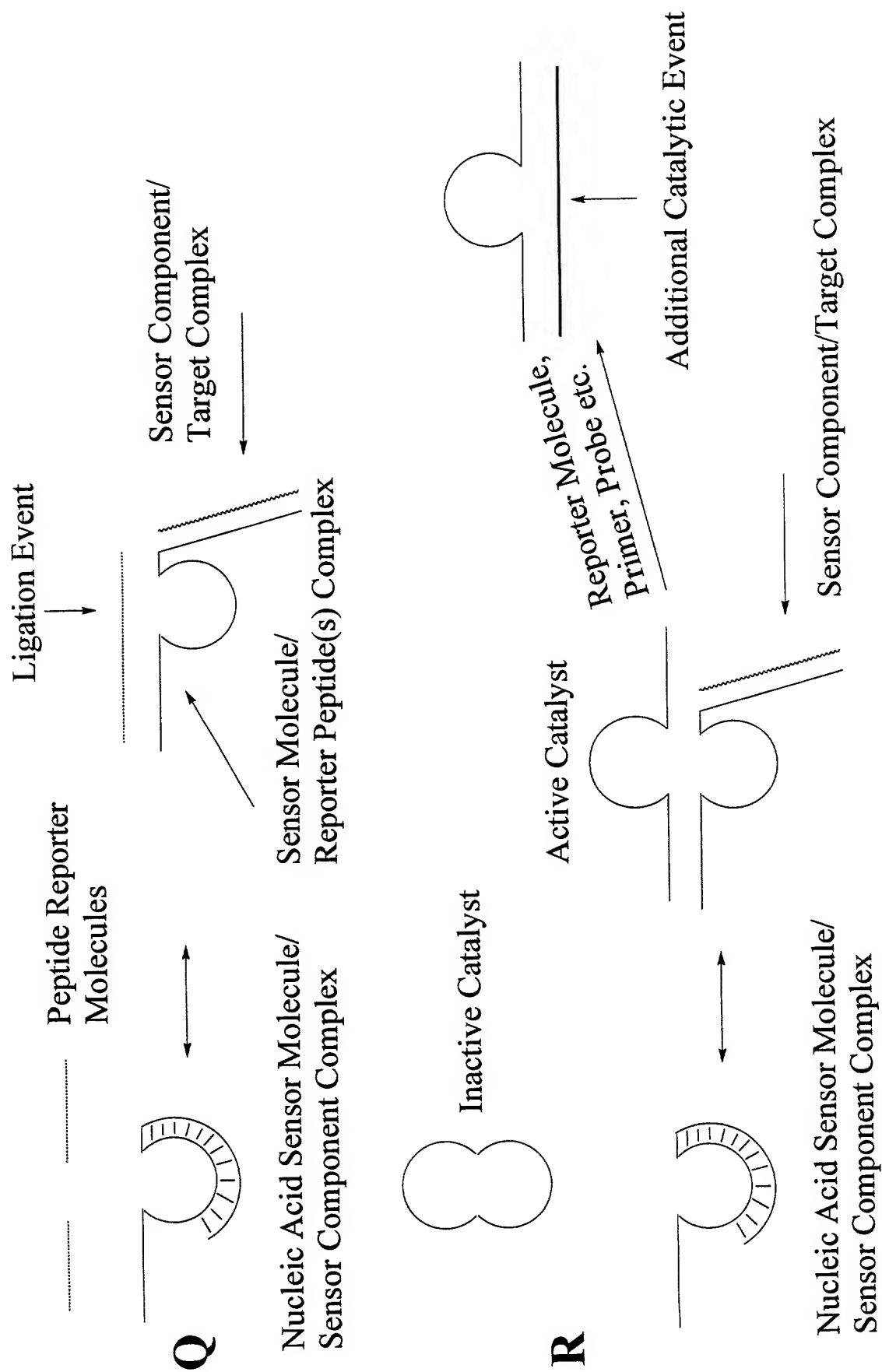


Figure 14: Inherent Amplification of Signal

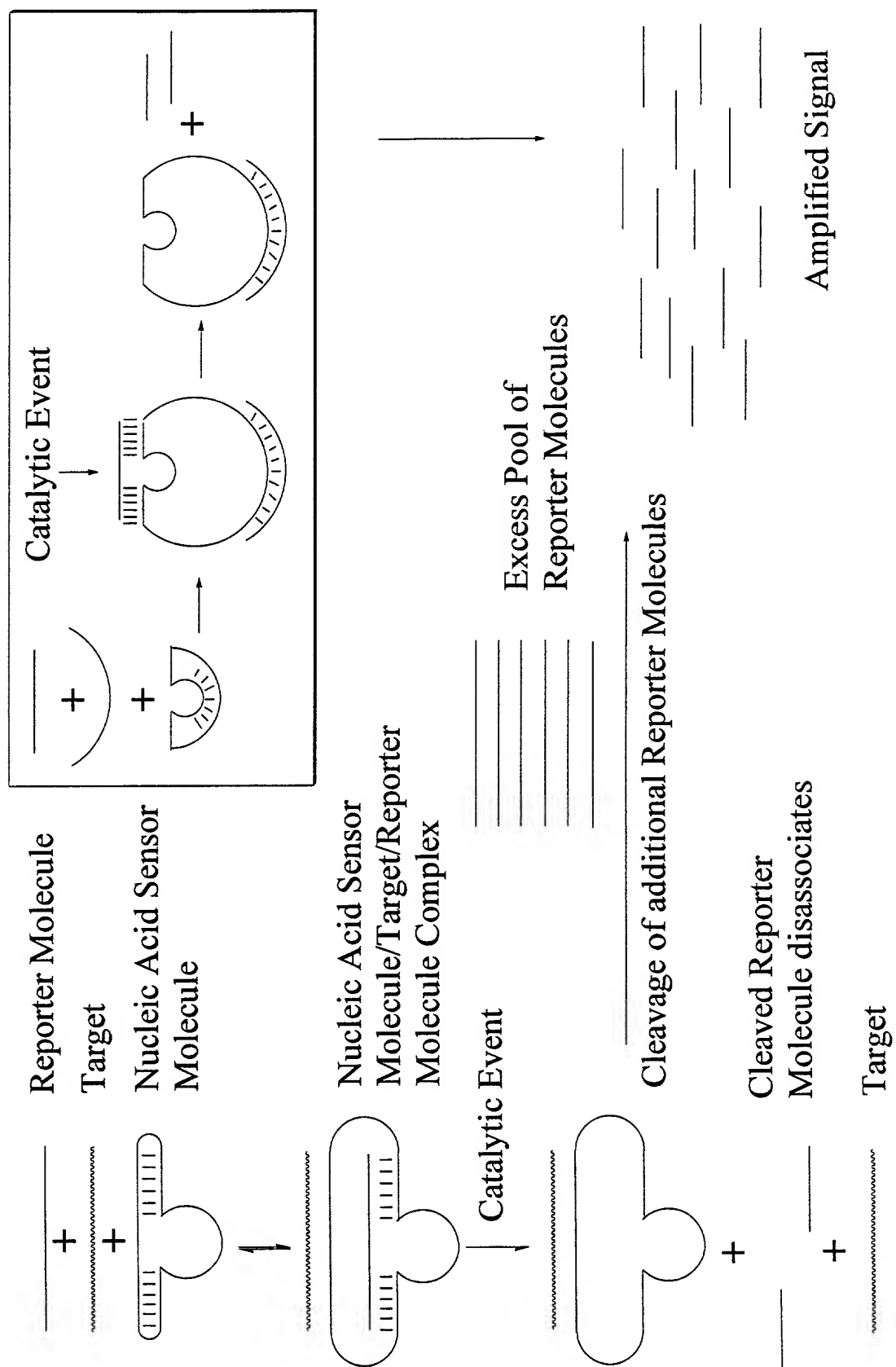


Figure 15: Example of Diagnostic System

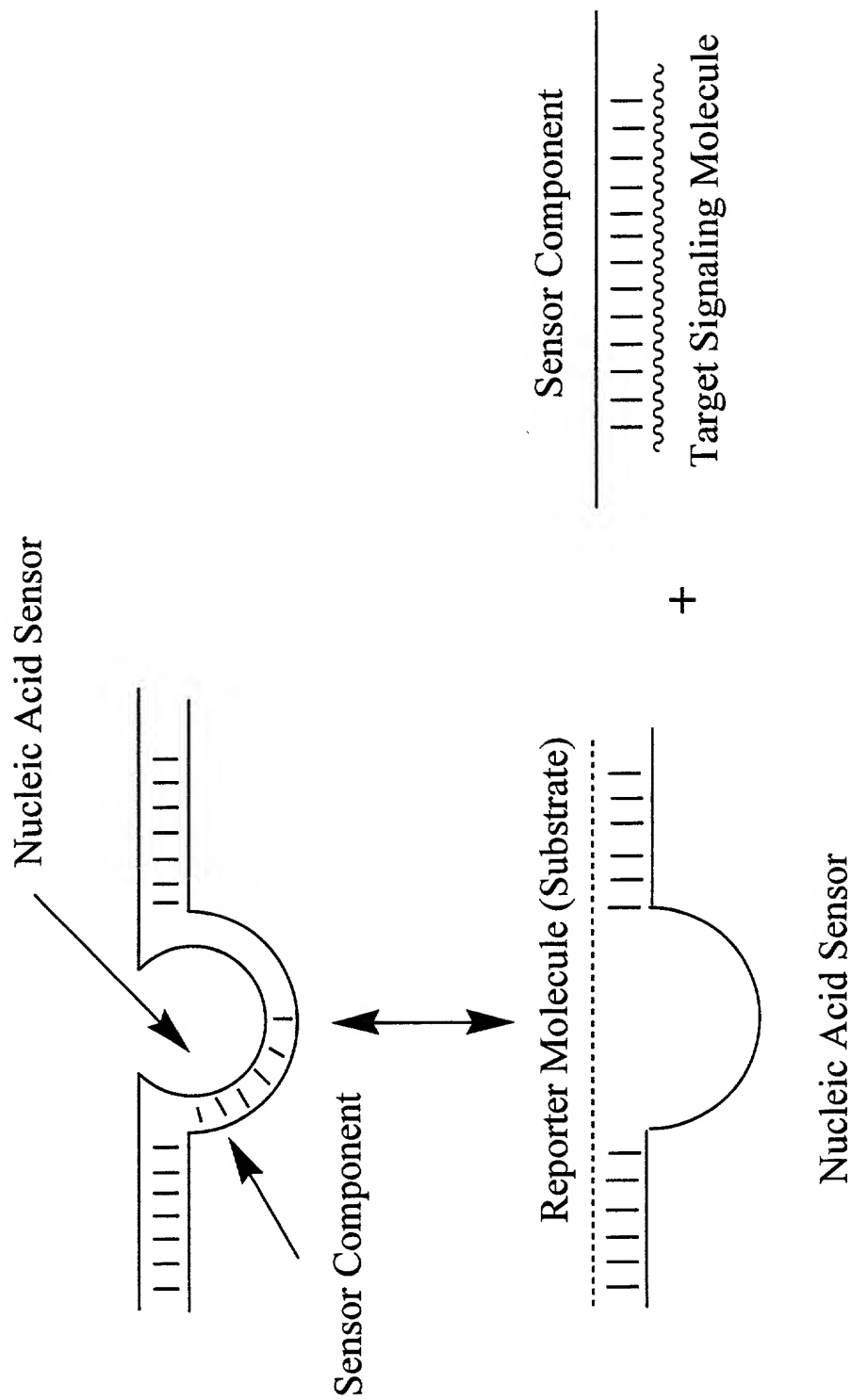


Figure 16: Ribozyme Diagnostic Screen
INHIBITORY FOLDING WITH TARGET RESCUE

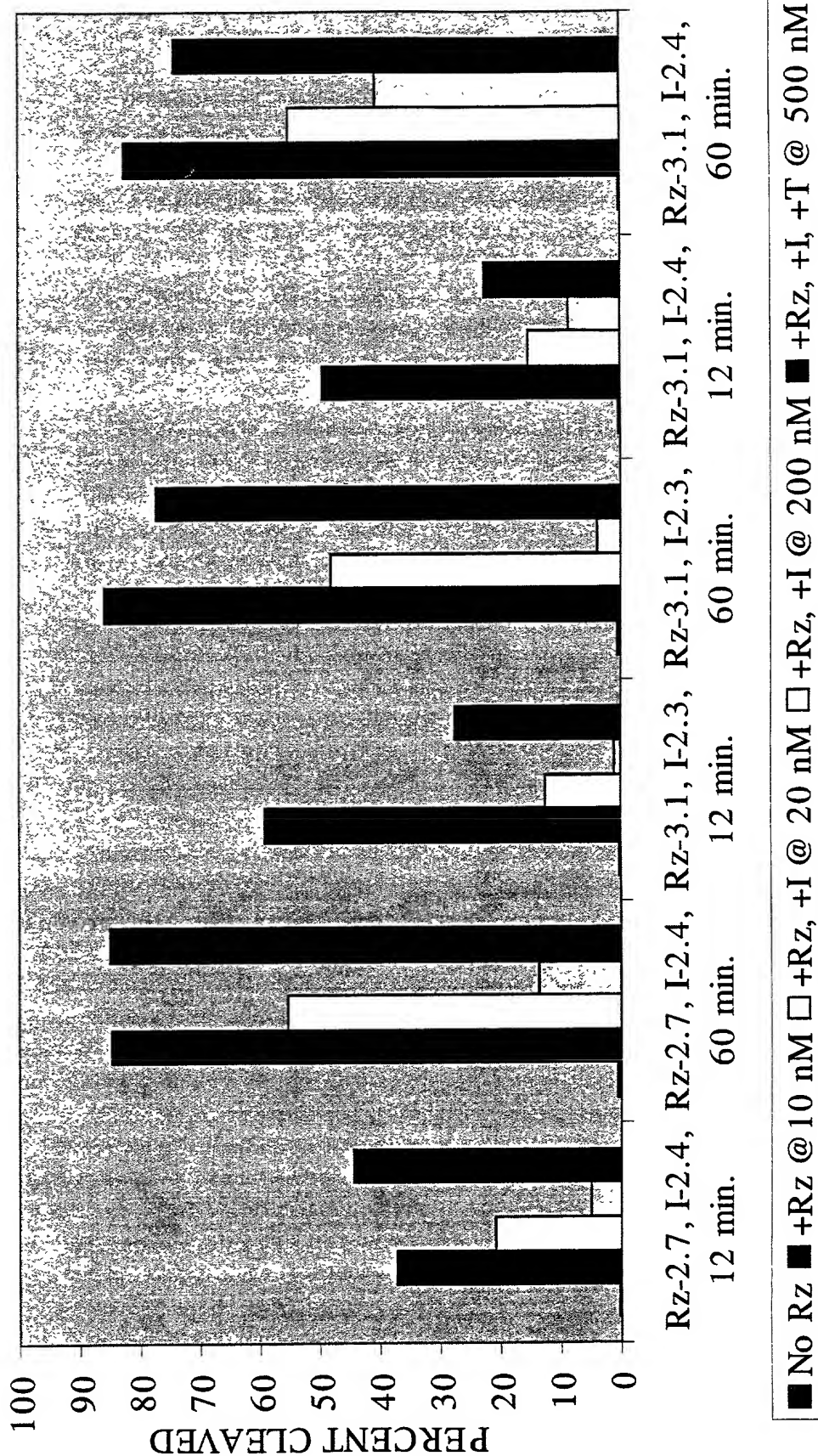
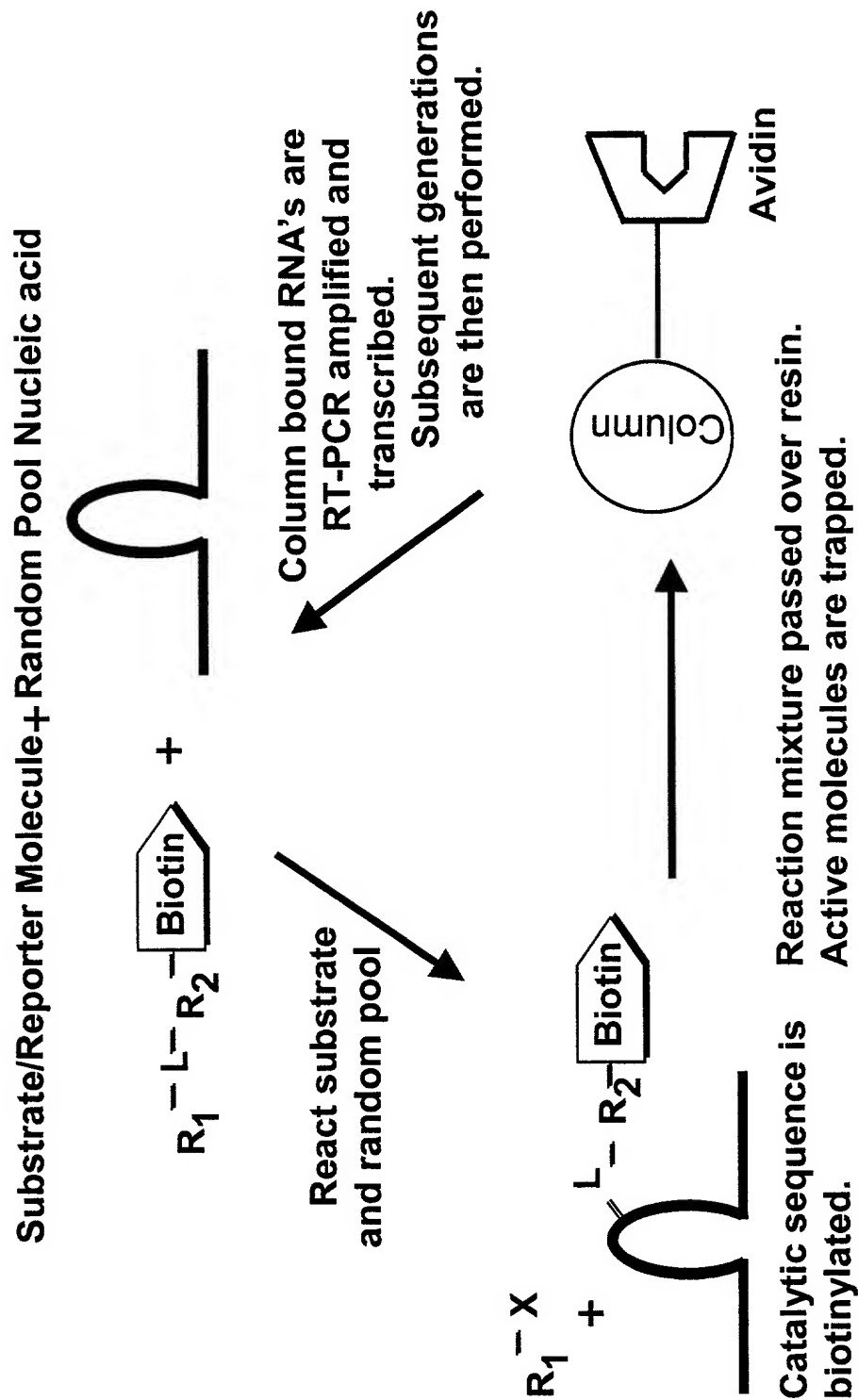
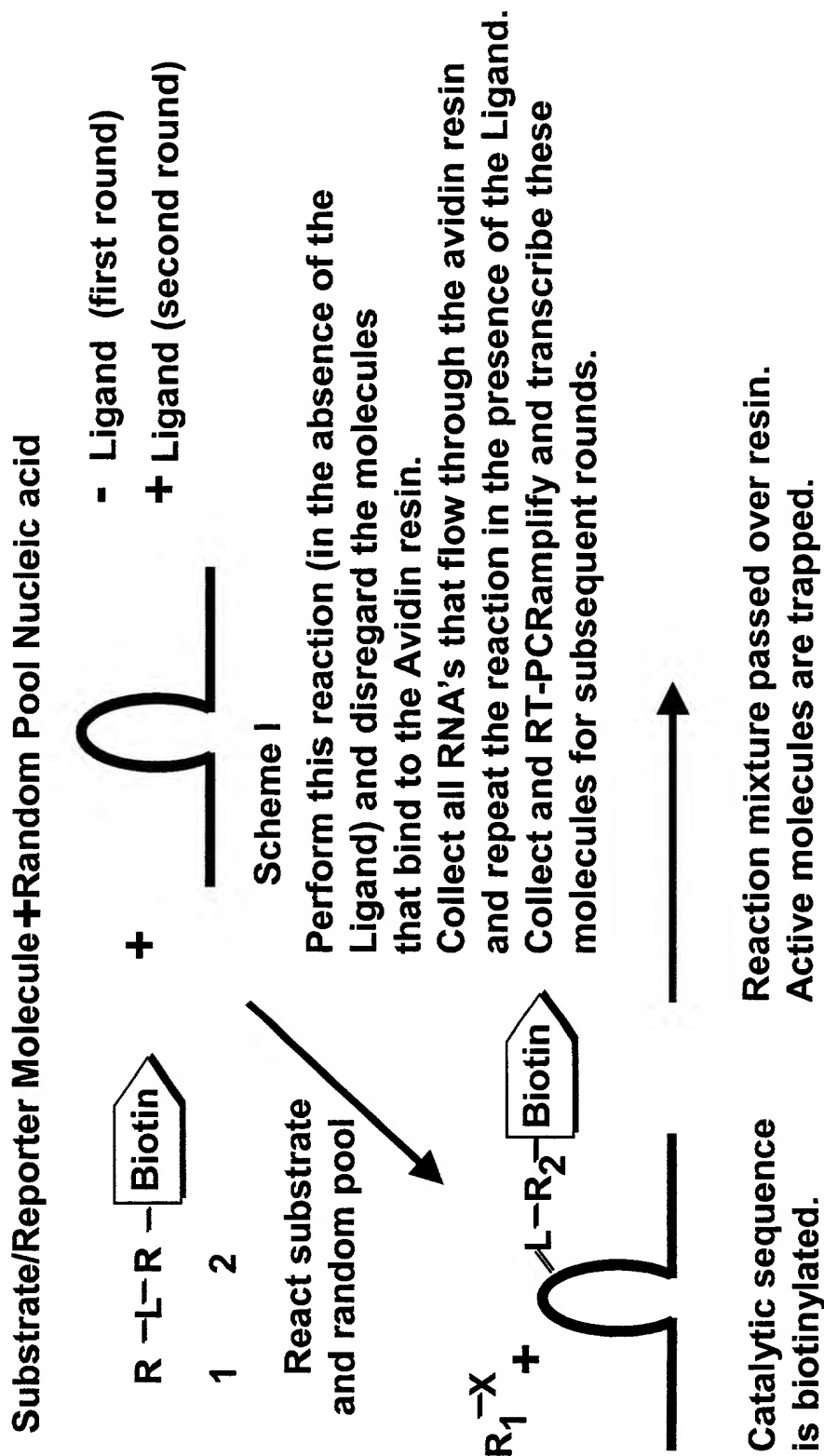


Figure 17a: Auto-ligation Nucleic Acid Sensor Molecules - Selection Scheme



**Figure 17b: Auto-ligation Nucleic Acid Sensor Molecules -
Ligand Dependent**



**Figure 17c: Auto-ligation Nucleic Acid Sensor Molecules-
Ligand dependent**

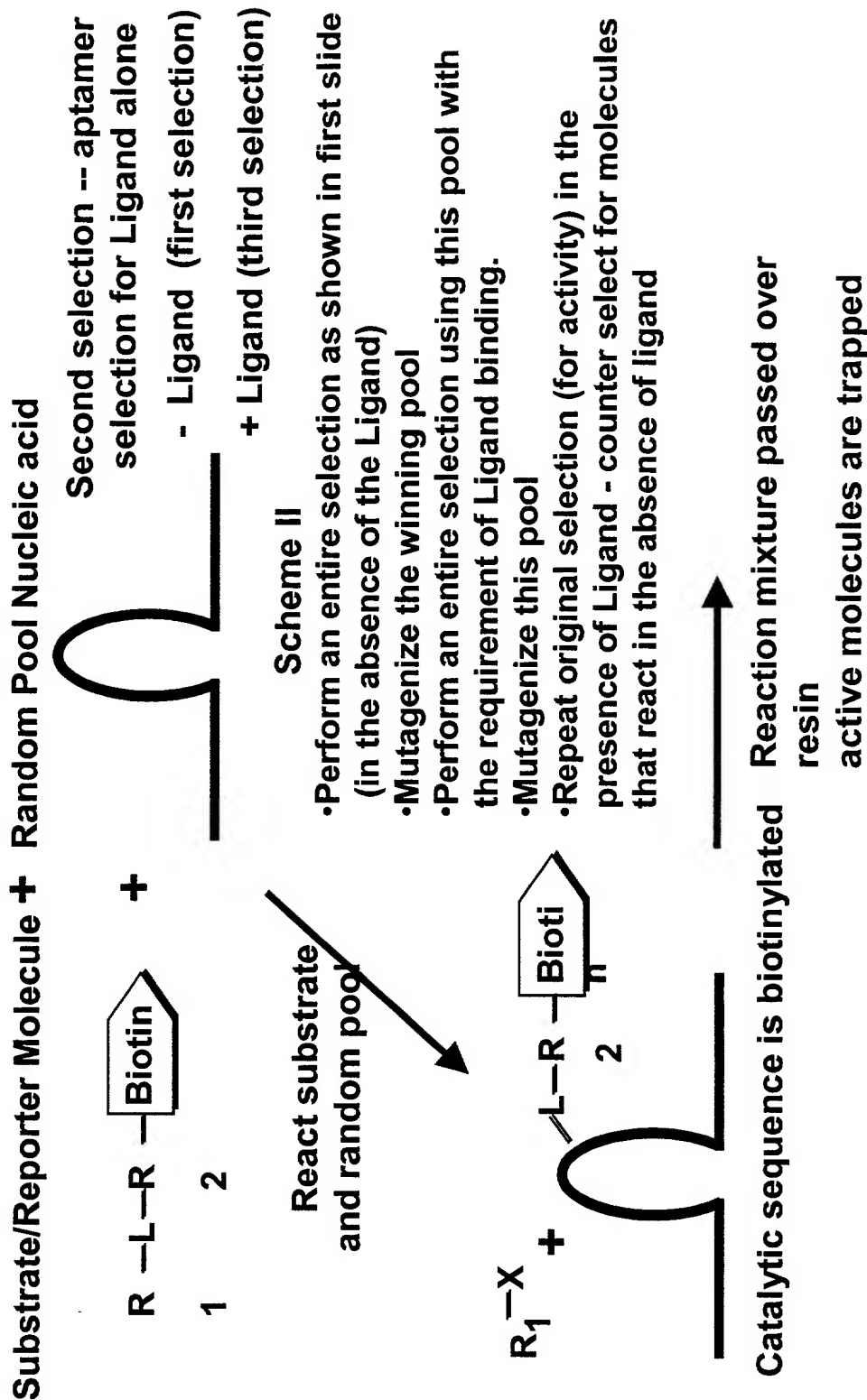


Figure 18a: Isomerase Nucleic Acid Sensor Molecule - Selection Scheme

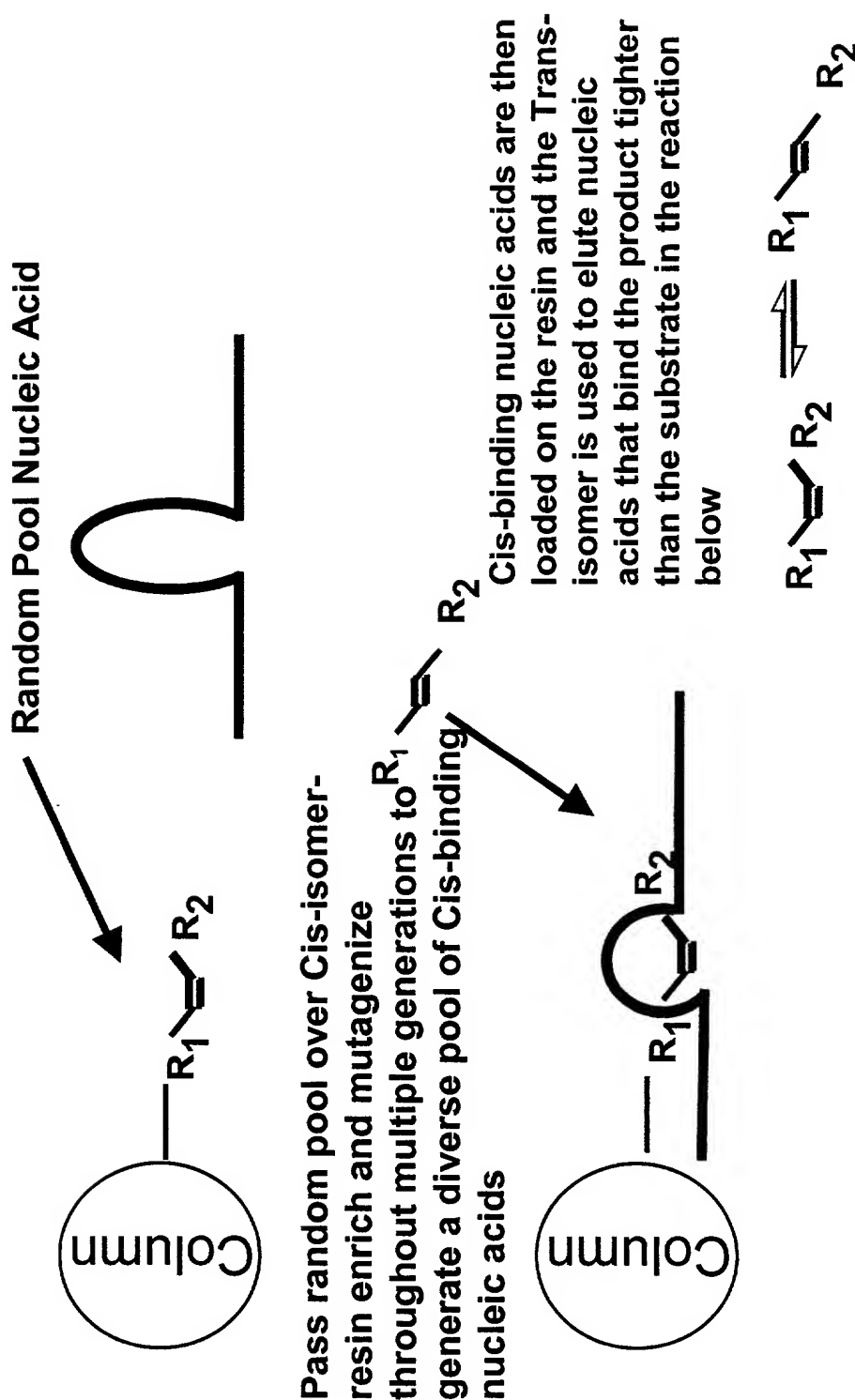
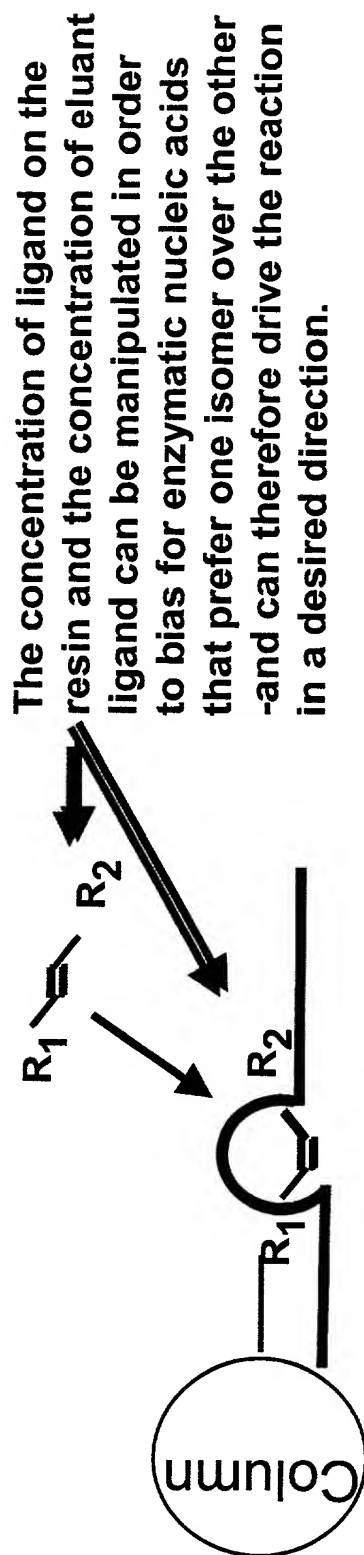


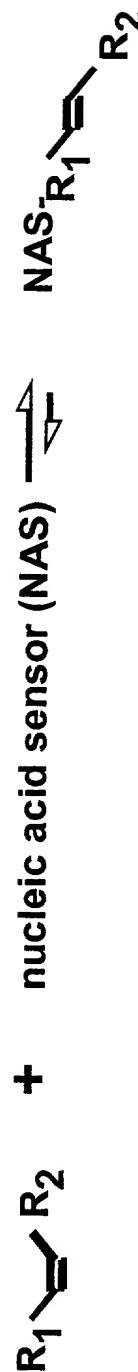
Figure 18b: Isomerase Nucleic Acid Sensor Molecule - Selection Scheme



E.g. Selection for Cis-isomer at 100 μM - yield $\text{cis } K_d = 100 \mu\text{M}$

Elute with Trans-isomer at 0.1 μM - yield $\text{trans } K_d = 0.1 \mu\text{M}$

Isolate catalysts for the reaction below



**Figure 18c: Isomerase Nucleic Acid Sensor Molecule -
Ligand dependent**

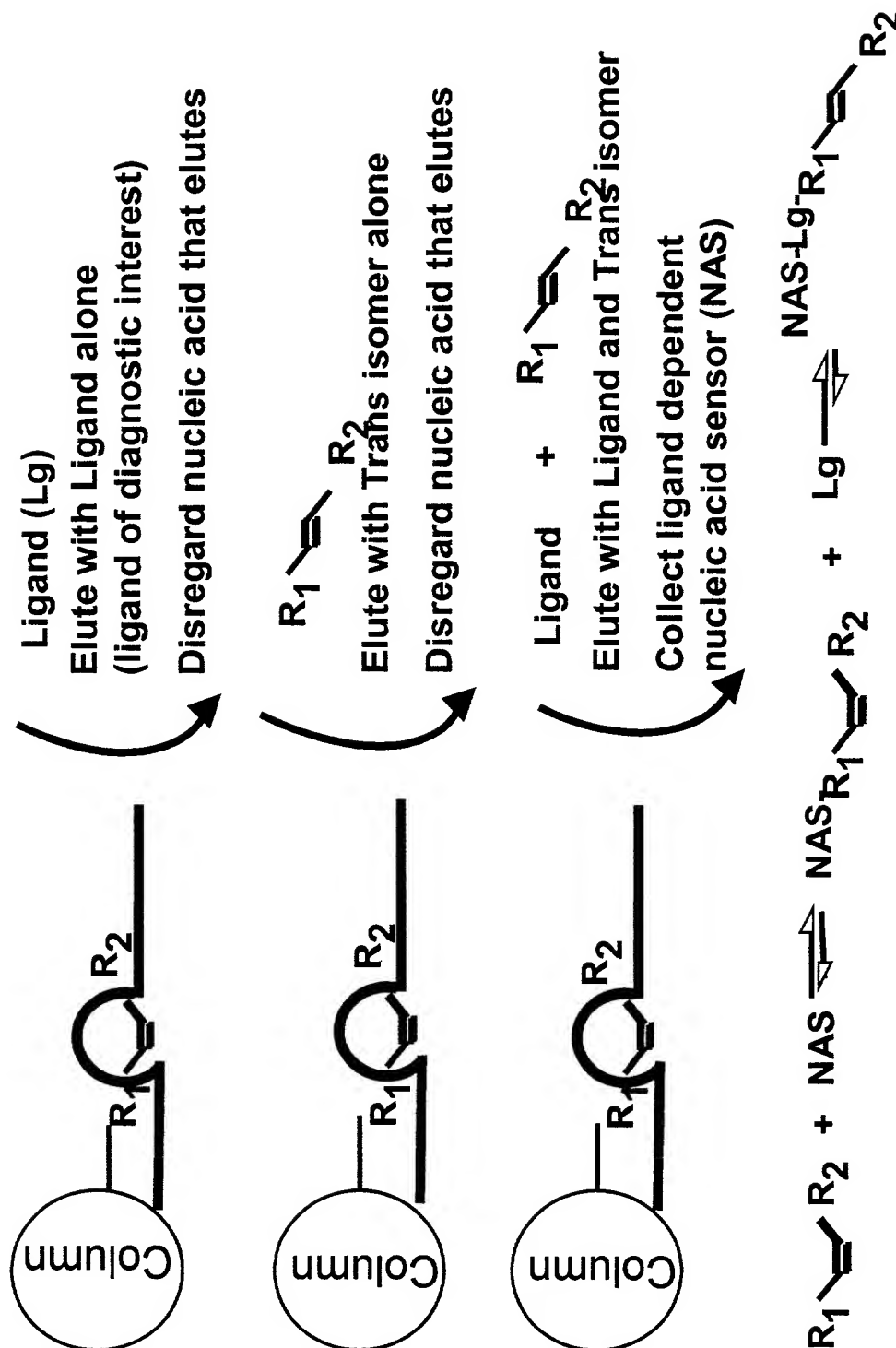
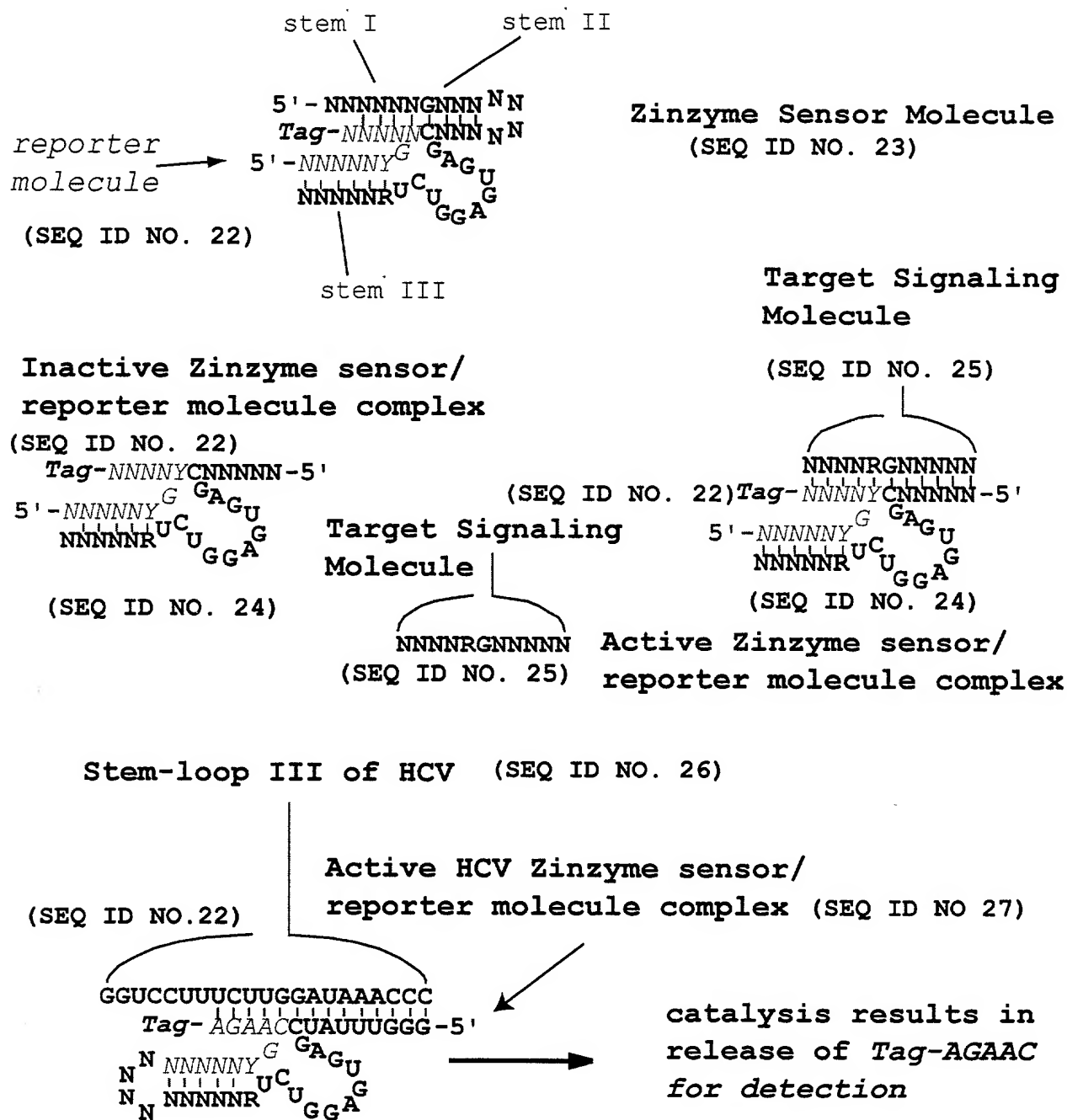


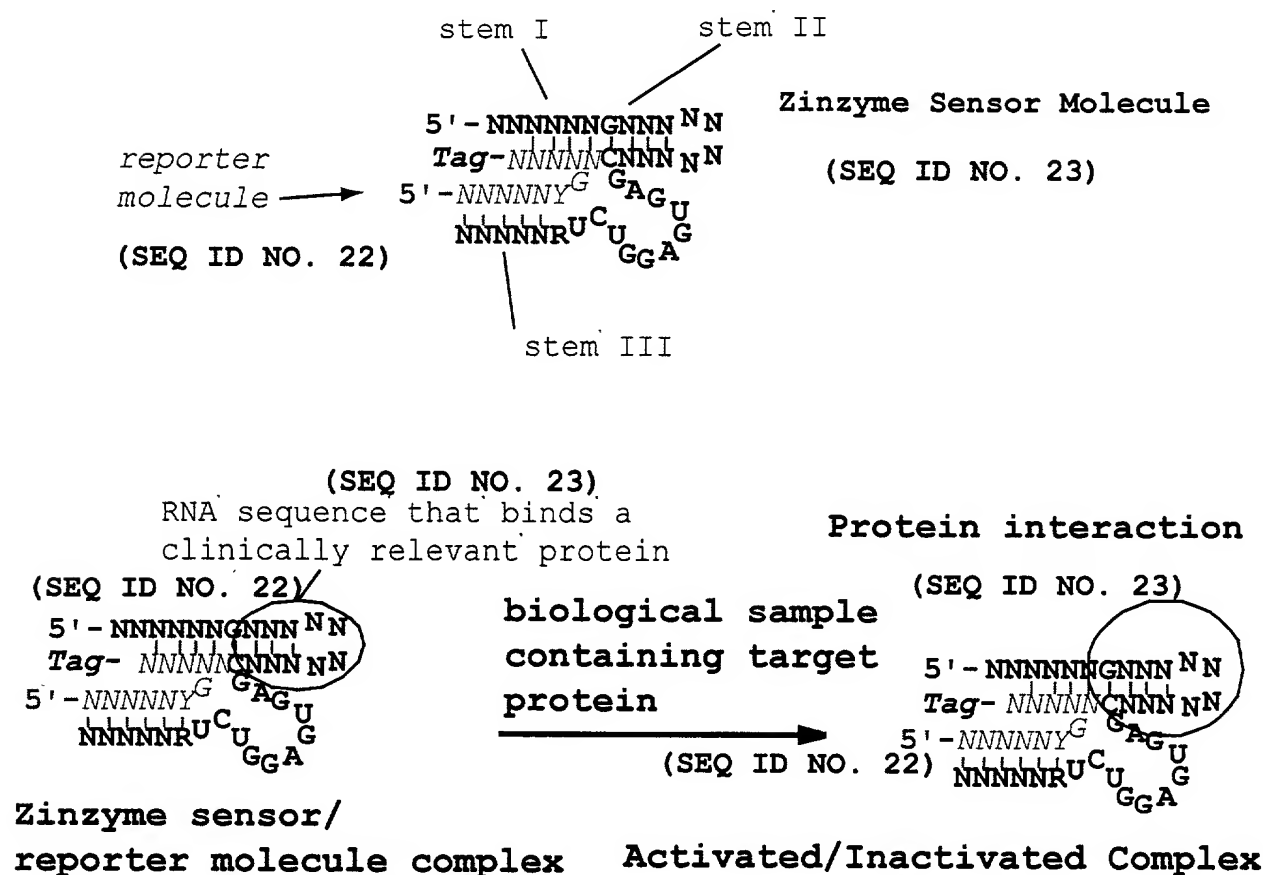
Figure 19: Zinzyme Sensor Molecule for detection of Nucleic Acid



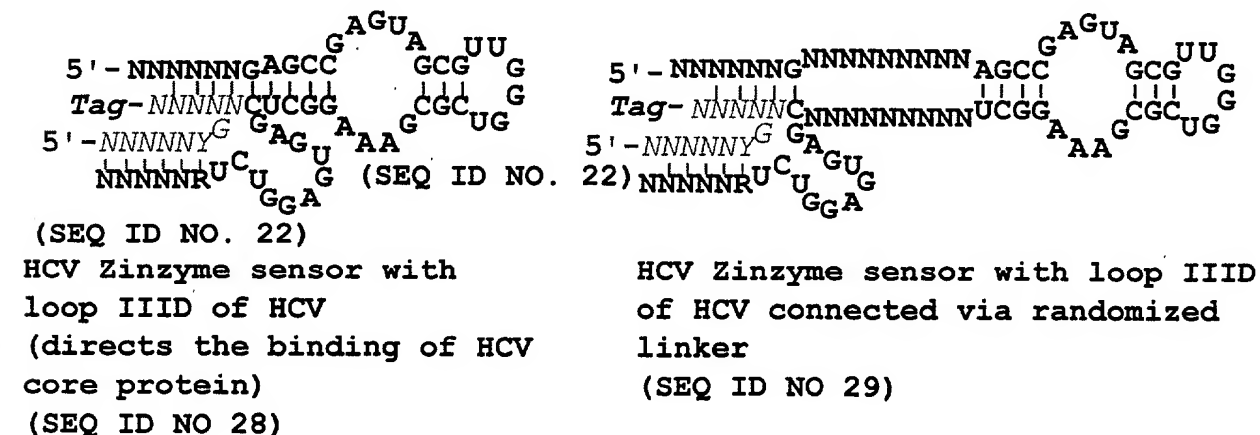
Zinzyme sensor can be attached to solid support/surface, for example at the 5'-end

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Figure 20: Zinzyme Sensor Molecule for detection of Protein



Sensor/reporter complex for detection of HCV core protein



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trans

high turnover protein enzyme

5' - NNNNNNGNNN NN
NNNNN CNNN NN
5' - NNNNNY G GAG U
NNNNNR U C U G
G G A

Reporter Molecule (SEQ ID NO. 22)
Zinzyme Sensor (SEQ ID NO. 23)

cis

high turnover protein enzyme

5' NNNNNNGNNN NN
NNNNN CNNN NN
NN NNNNNY G GAG U
NN NNNNNNR U C U G
G G A

Reporter Molecule (SEQ ID NO. 22)
Zinzyme Sensor (SEQ ID NO. 30)

RO

Base

HO HO

Sodium Periodate

RO

Base

a. Protein
b. Sodium Borohydride

RO

Base

Protein Enzyme

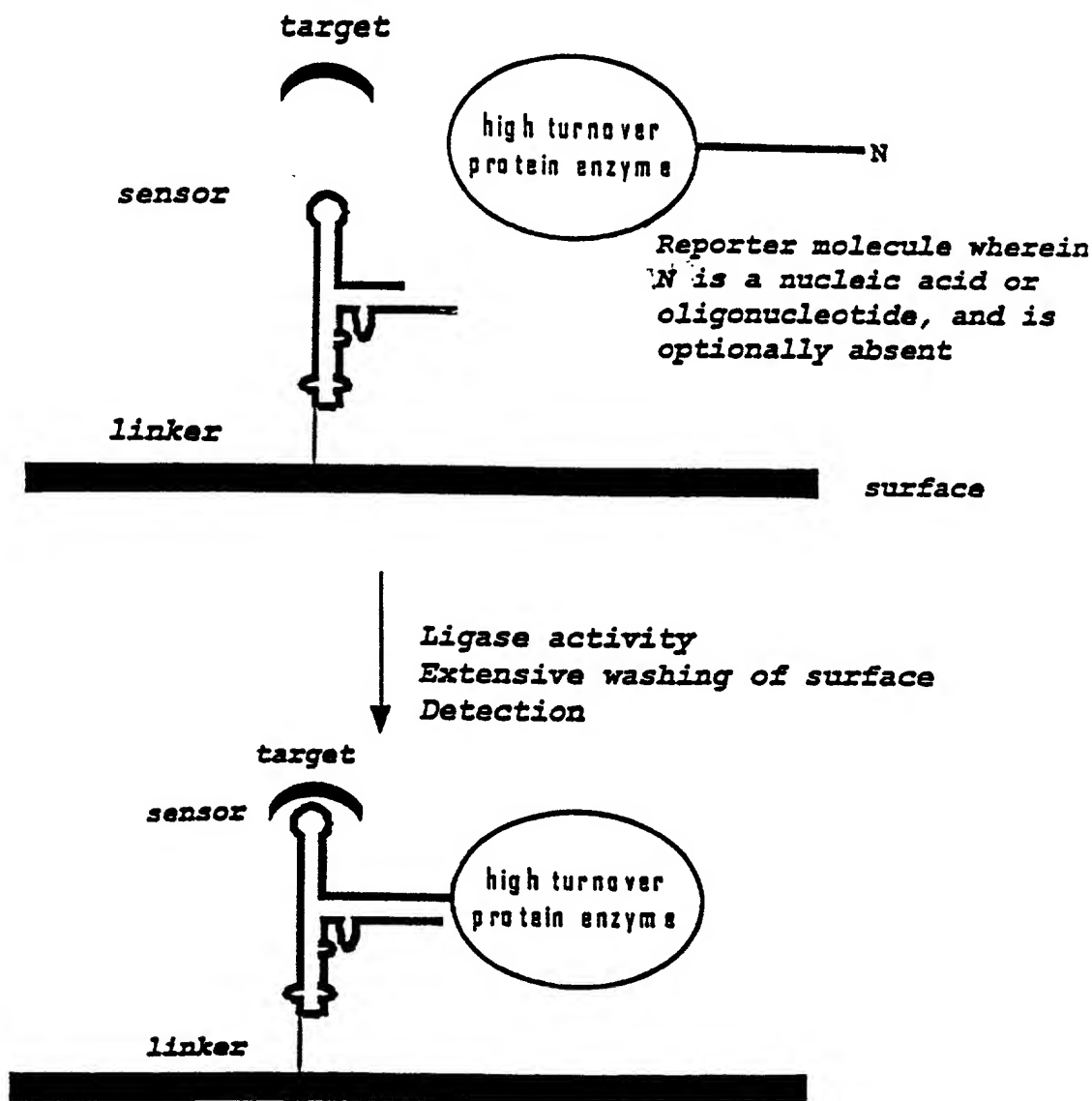
Protein can be attached via amino linker.

Alternately, R is phosphoramidite moiety for incorporation at 5'-end of oligonucleotide.

High turnover protein enzyme is, for example, Luciferase, Horseradish peroxidase, beta-galactosidase, alkaline phosphatase.

Figure 22: Amplification of signal via use of protein enzyme conjugate

Ligase Sensor Molecule with enzymatic reporter



Alternatively, a fluorescent or chemiluminescent based reporter molecule is used.

FIG. 23

Figure 24: Selection of Nucleic Acid Sensor Molecules with Ligase Activity

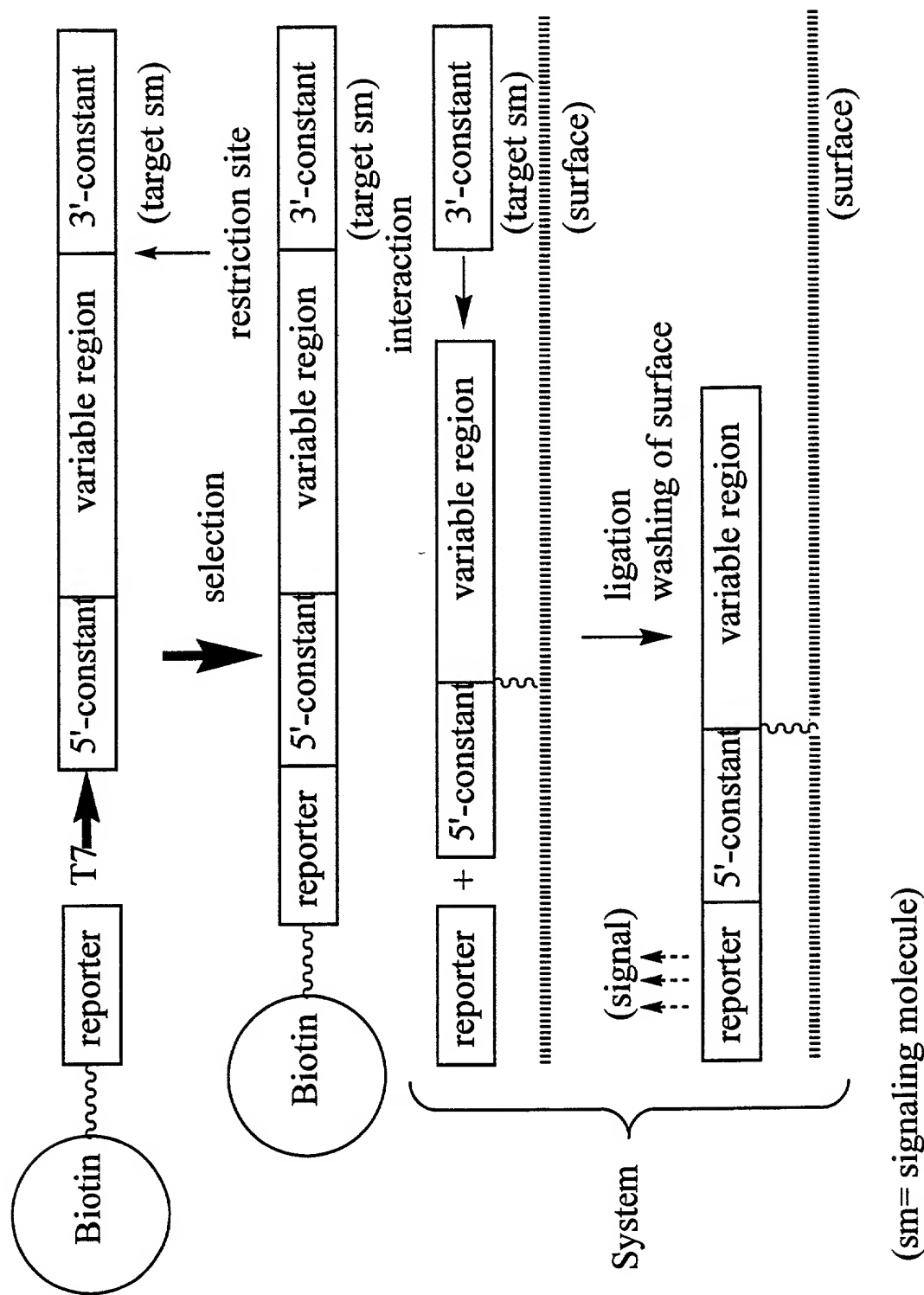


Figure 25: Nucleic Acid Sensor Molecule-Based Electric Circuit

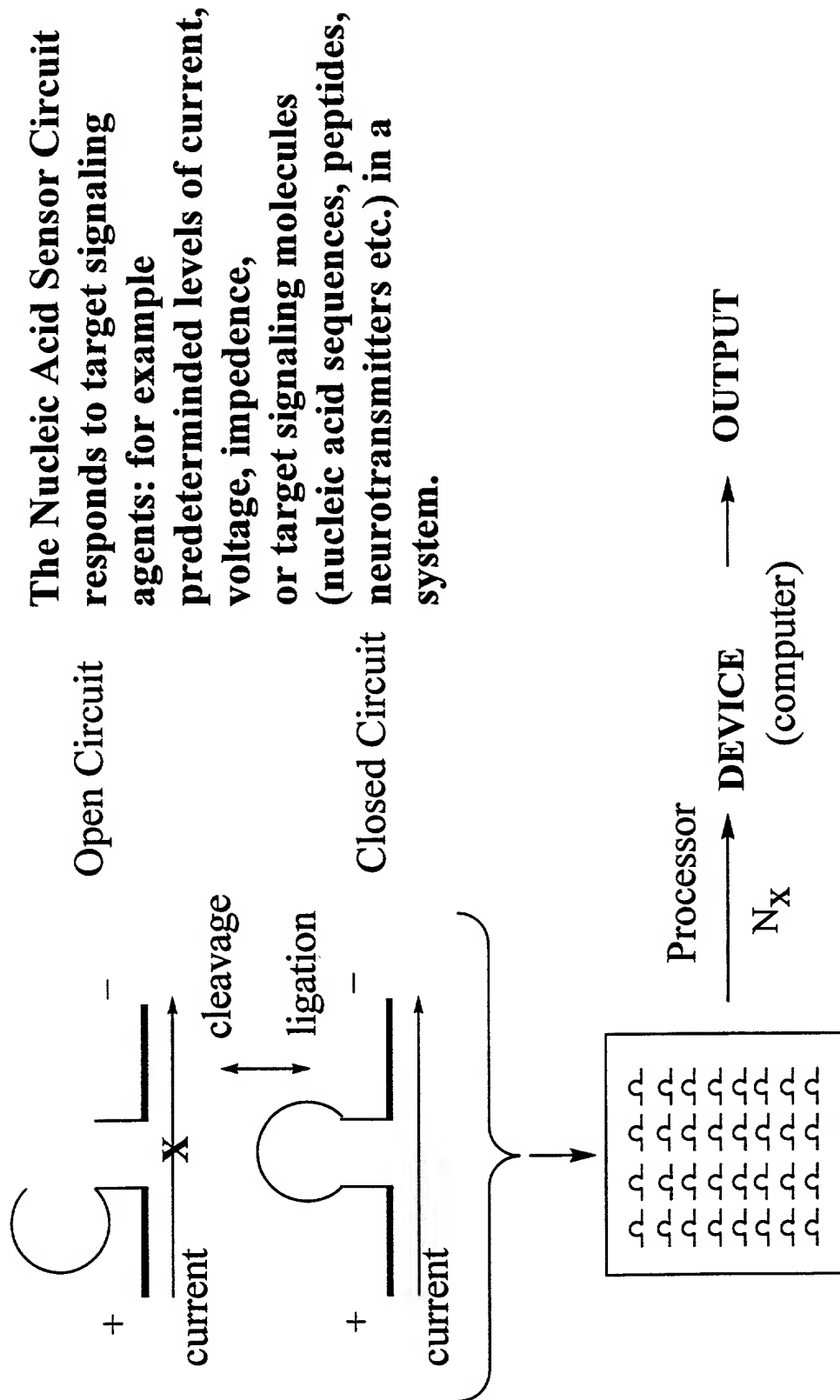
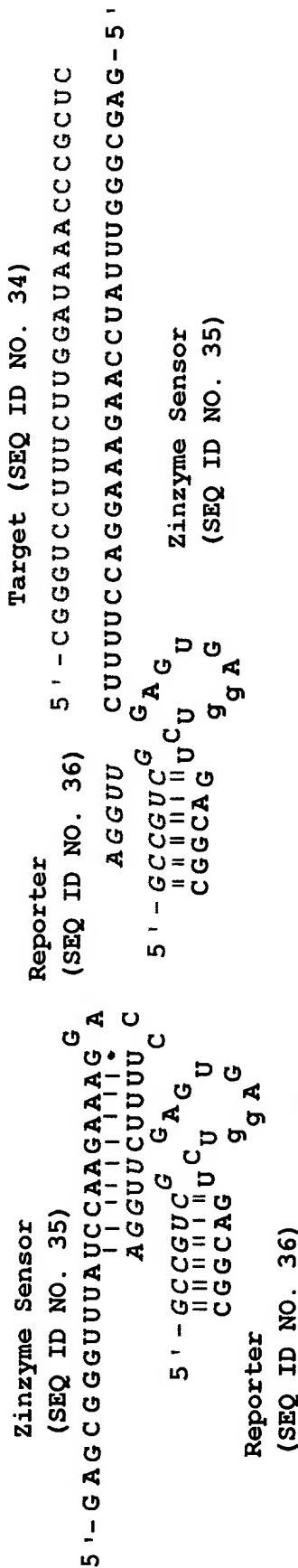


Figure 26: Target Inactivation of Zinzyme Sensor Molecule



ACTIVE ↔ TARGET INACTIVATED

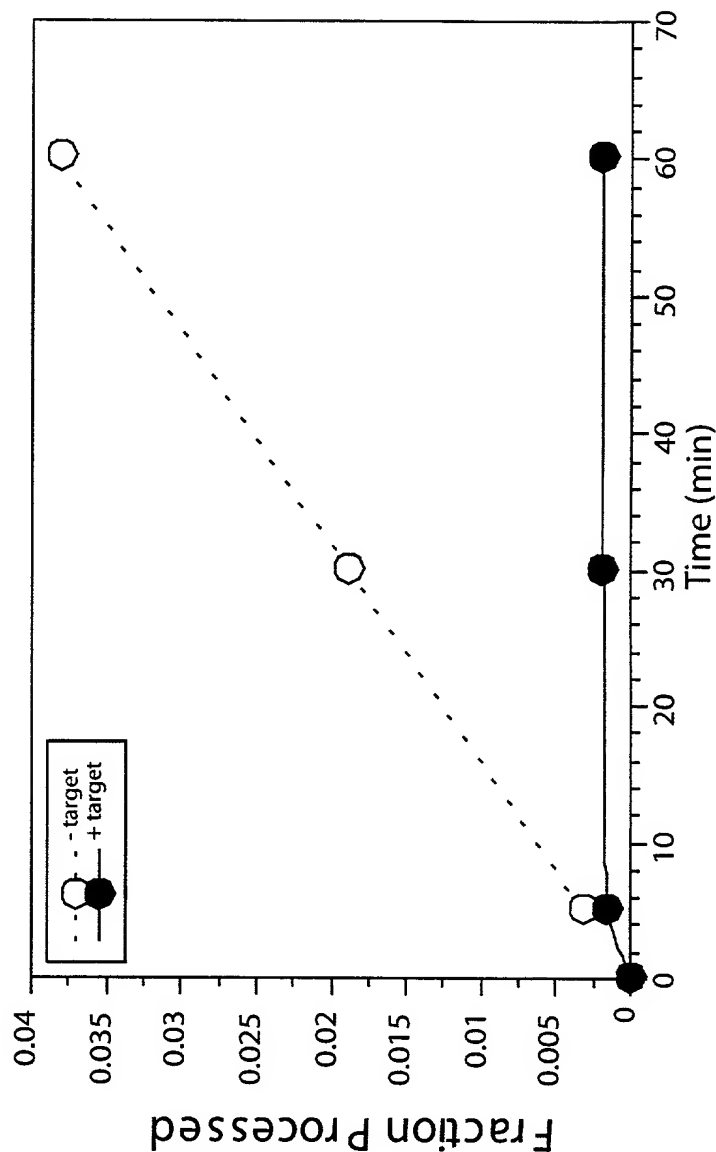
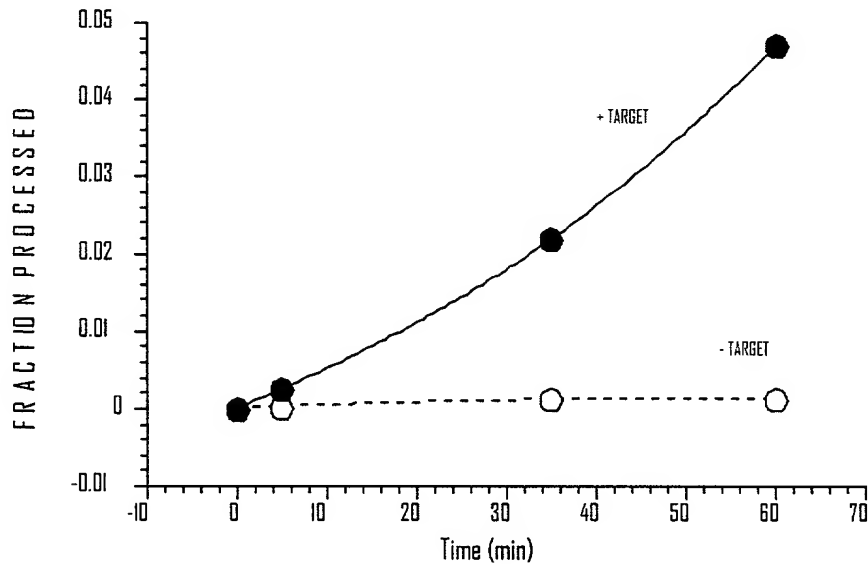
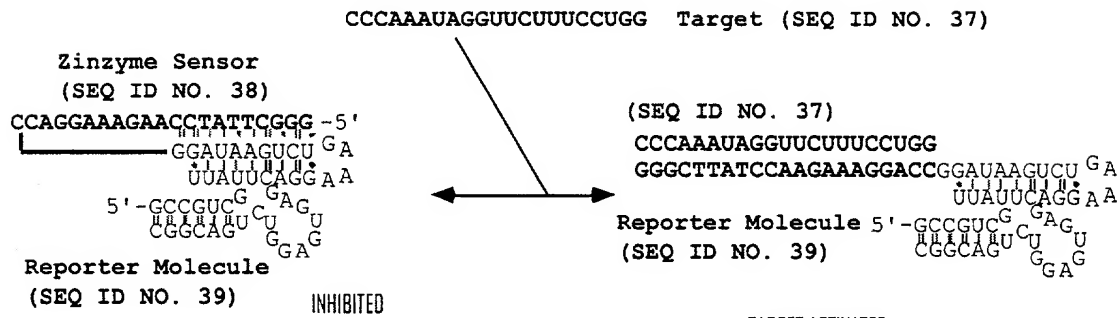


Figure 27: Target Activation of Zinzyme Sensor Molecule



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Figure 28: *Erk* modulated Nucleic Acid Sensor Molecule

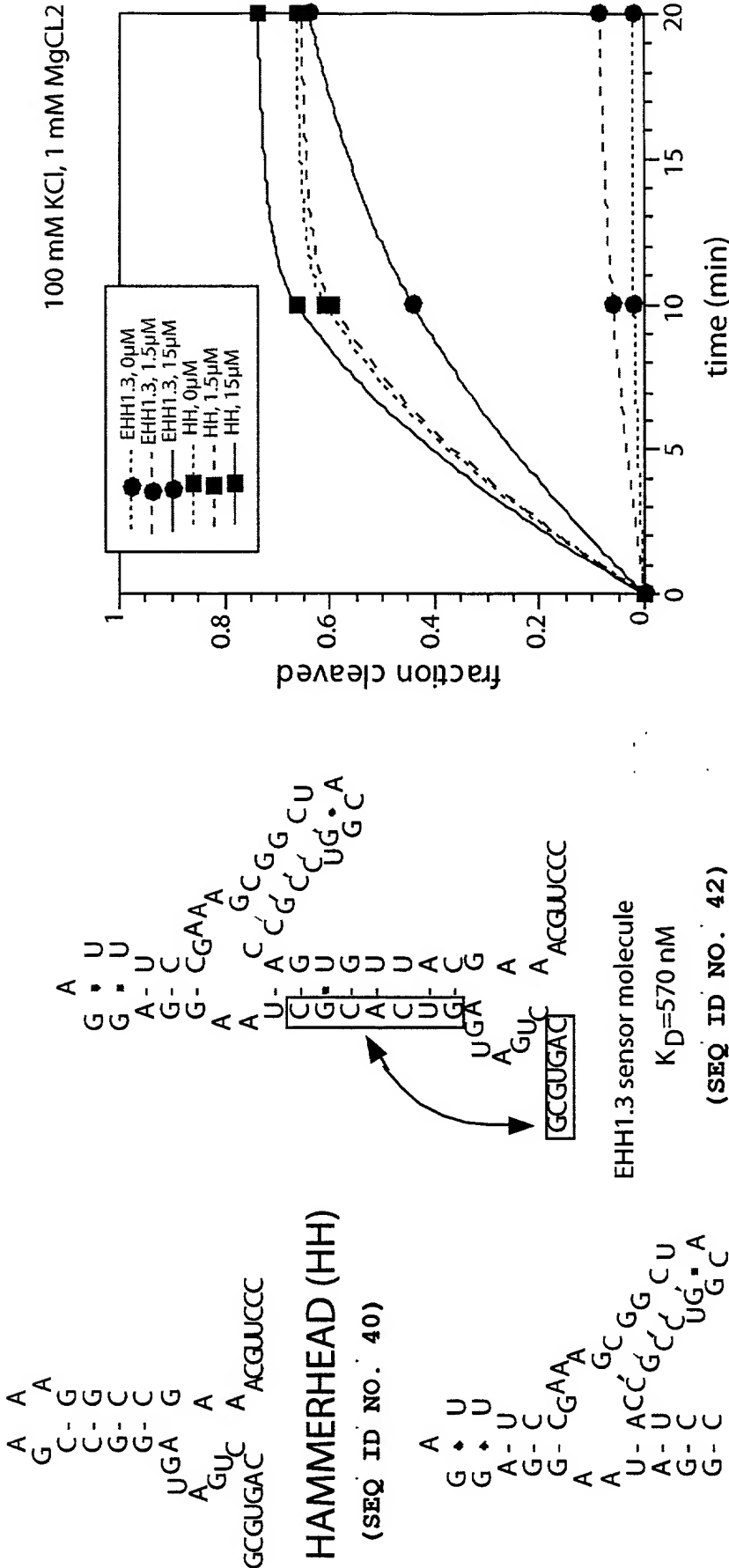
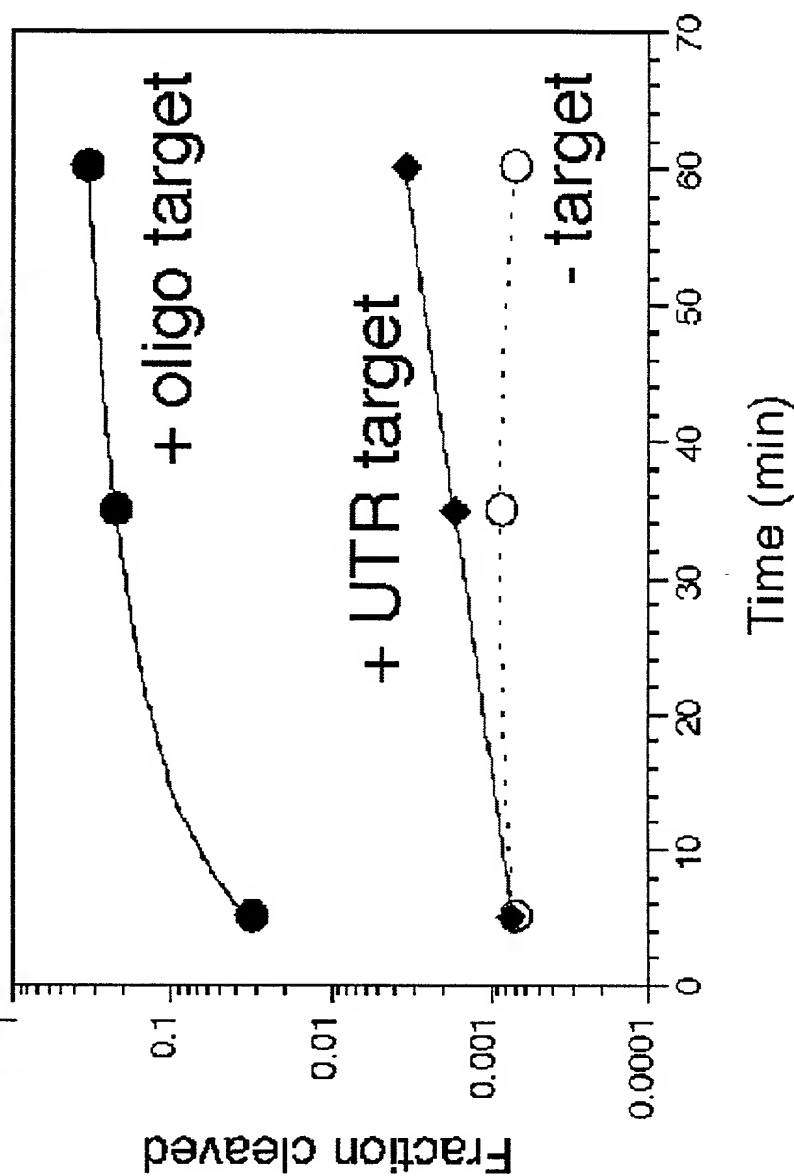


Diagram illustrating the cleavage mechanism of a target signaling molecule:

Inactive: The molecule consists of a sequence `ggaaagaagCuaau-5' TARGET` followed by a **Half-Zinczyme (SEQ ID NO. 42)**. A **PEG linker** is attached to the sequence. An arrow labeled **Cleavage site** points to the `C` in the sequence.

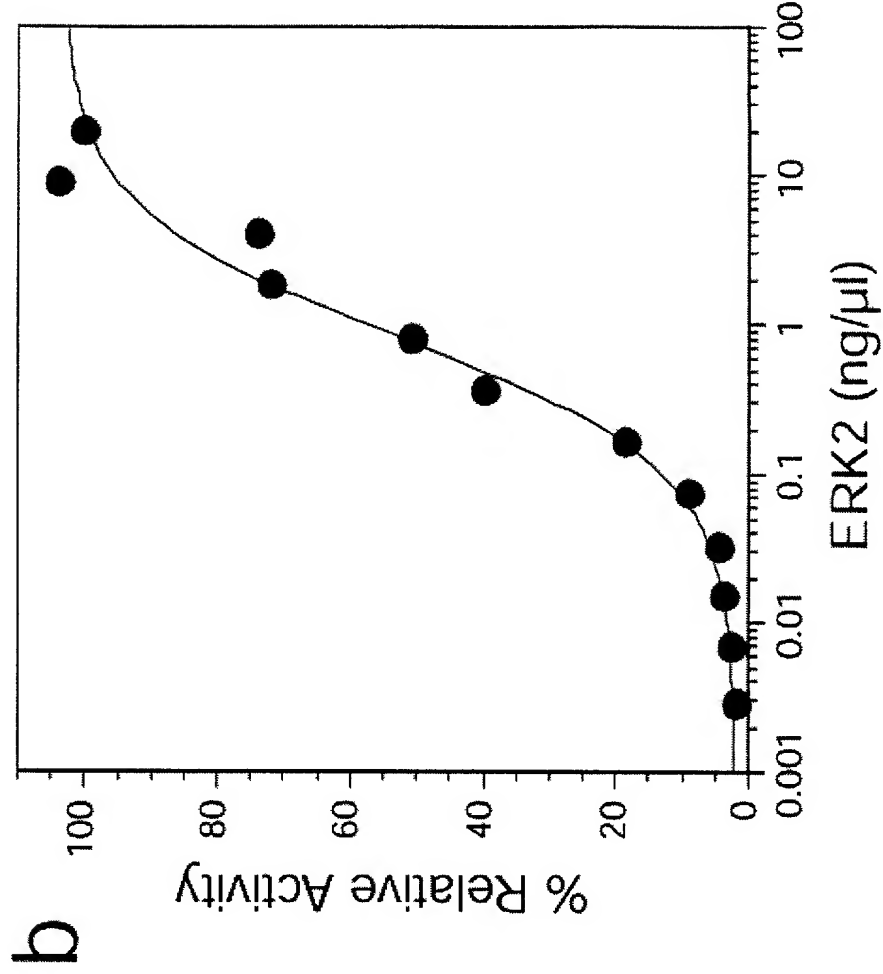
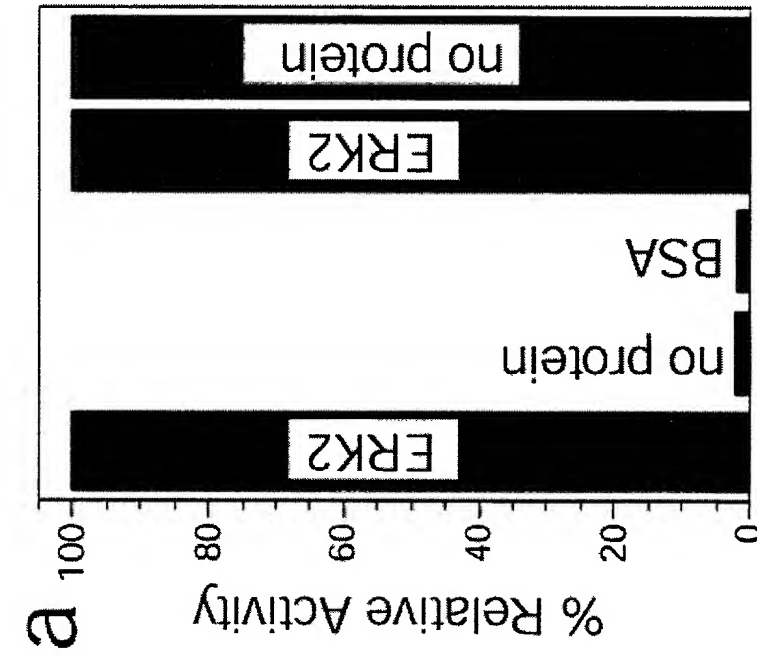
Active: The molecule is cleaved, resulting in the sequence `GGUCCUUUCUGAUA` and the **Half-Zinczyme (SEQ ID NO. 42)**. The resulting molecule is labeled **Active**.



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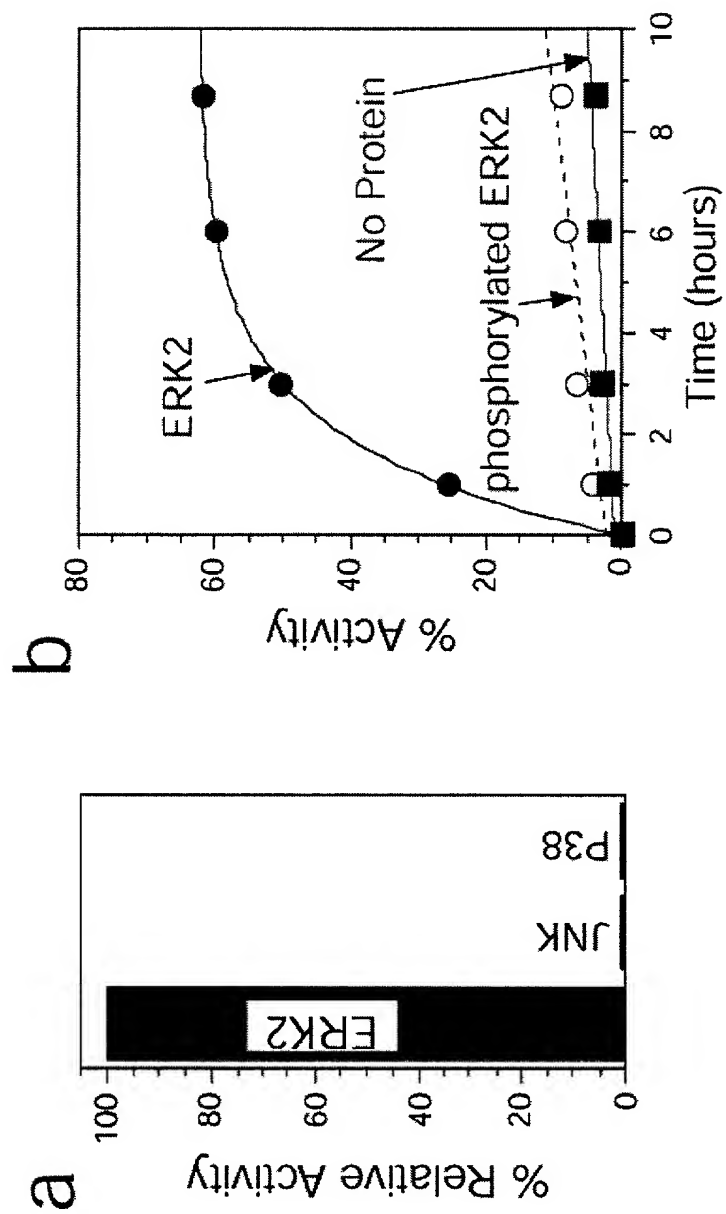


Figure 31



1005671E1.01E02

Figure 32



20221019125007

[illegible]

(SEQ ID NO 47)

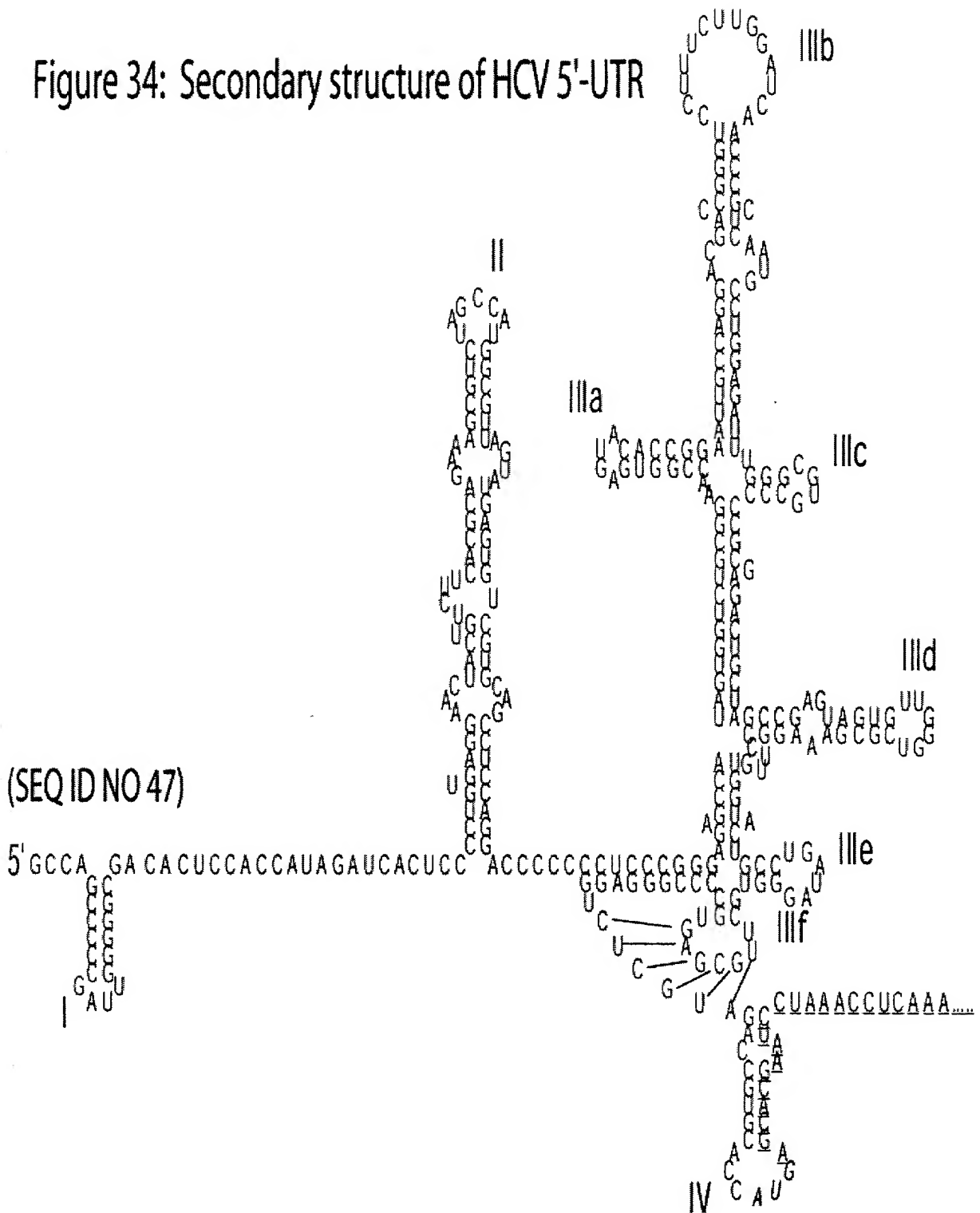


Figure 35
Design of SNP Detection using Halfzyme-AZB7.1

		SEQ ID NO:	
		aG G	
		g u g a g	
		AZB7.1	
		5'-a g c g C	
		Target HBV 1887(True)=AZB7-GG 3'-T C G C G	
		AZB7-AG 3'-T C G C A	
		AZB7-TG 3'-T C G C T	
		AZB7-CG 3'-T C G C C	
		AZB7-GA 3'-T C G C G	
		AZB7-GT 3'-T C G C G	
		AZB7-GC 3'-T C G C G	
		RNA HBV 1433 3'-U C G C G	
		58	

Figure 36: Single Nucleotide Polymorphism (SNP) Detection

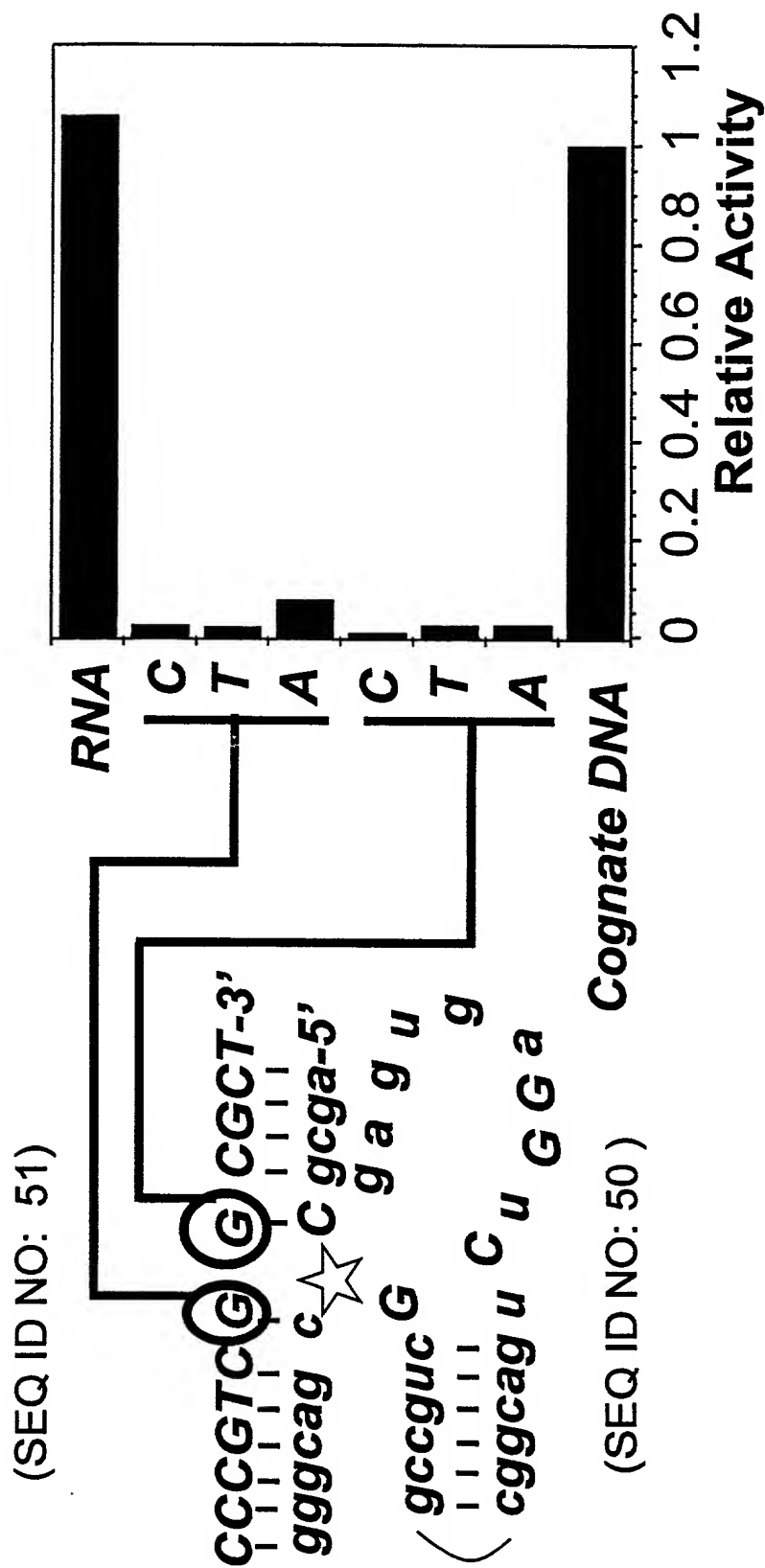
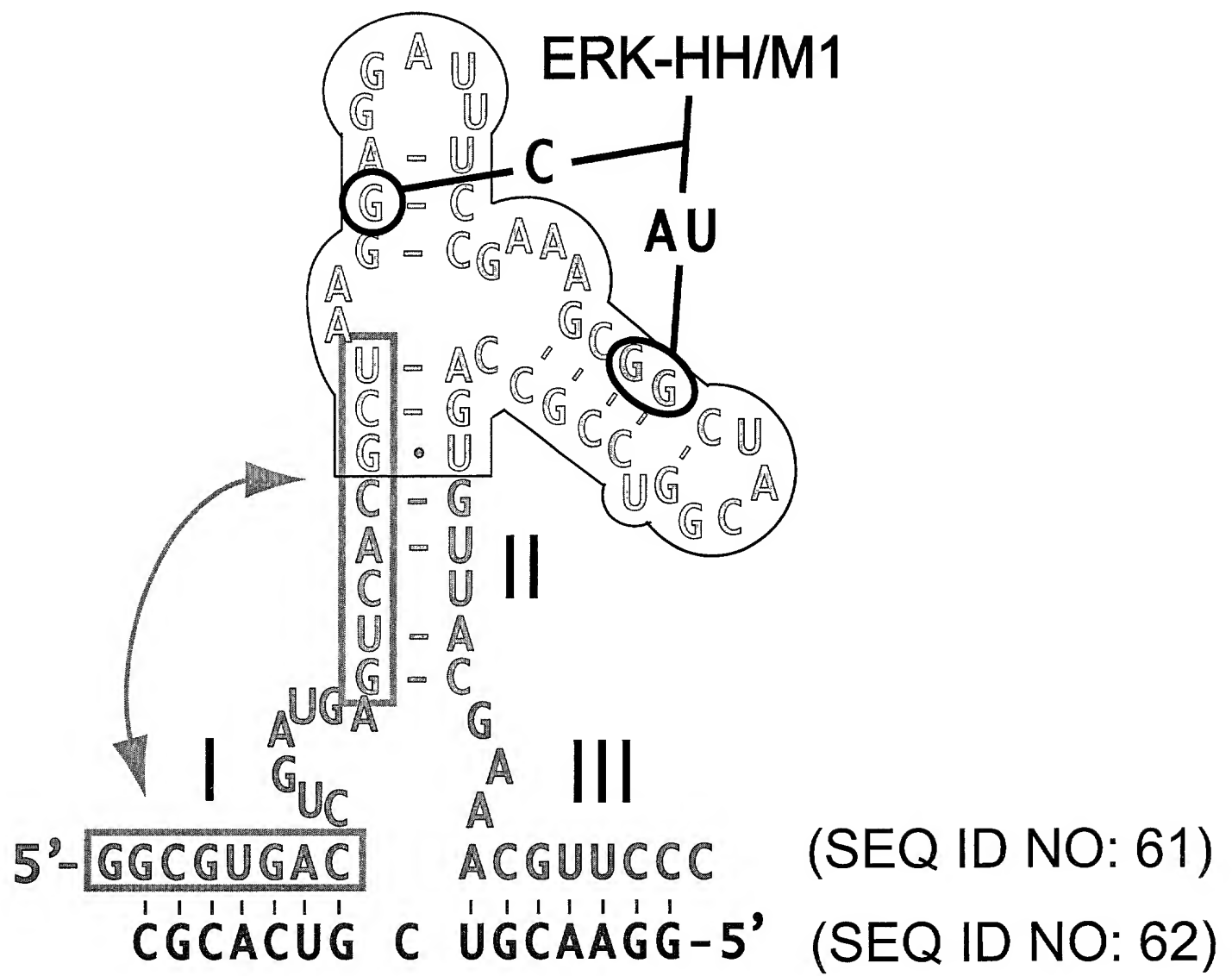
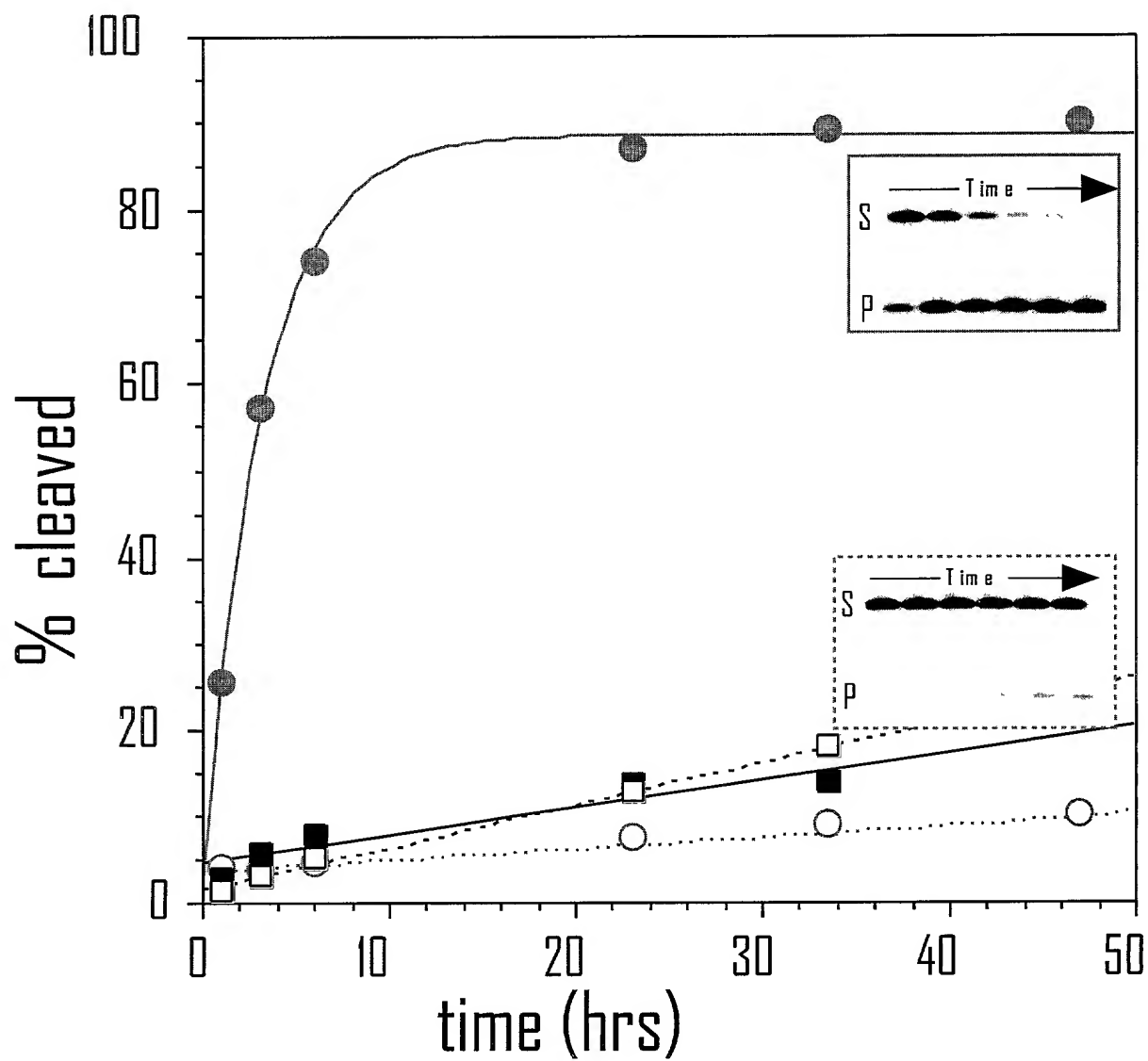


Figure 37A



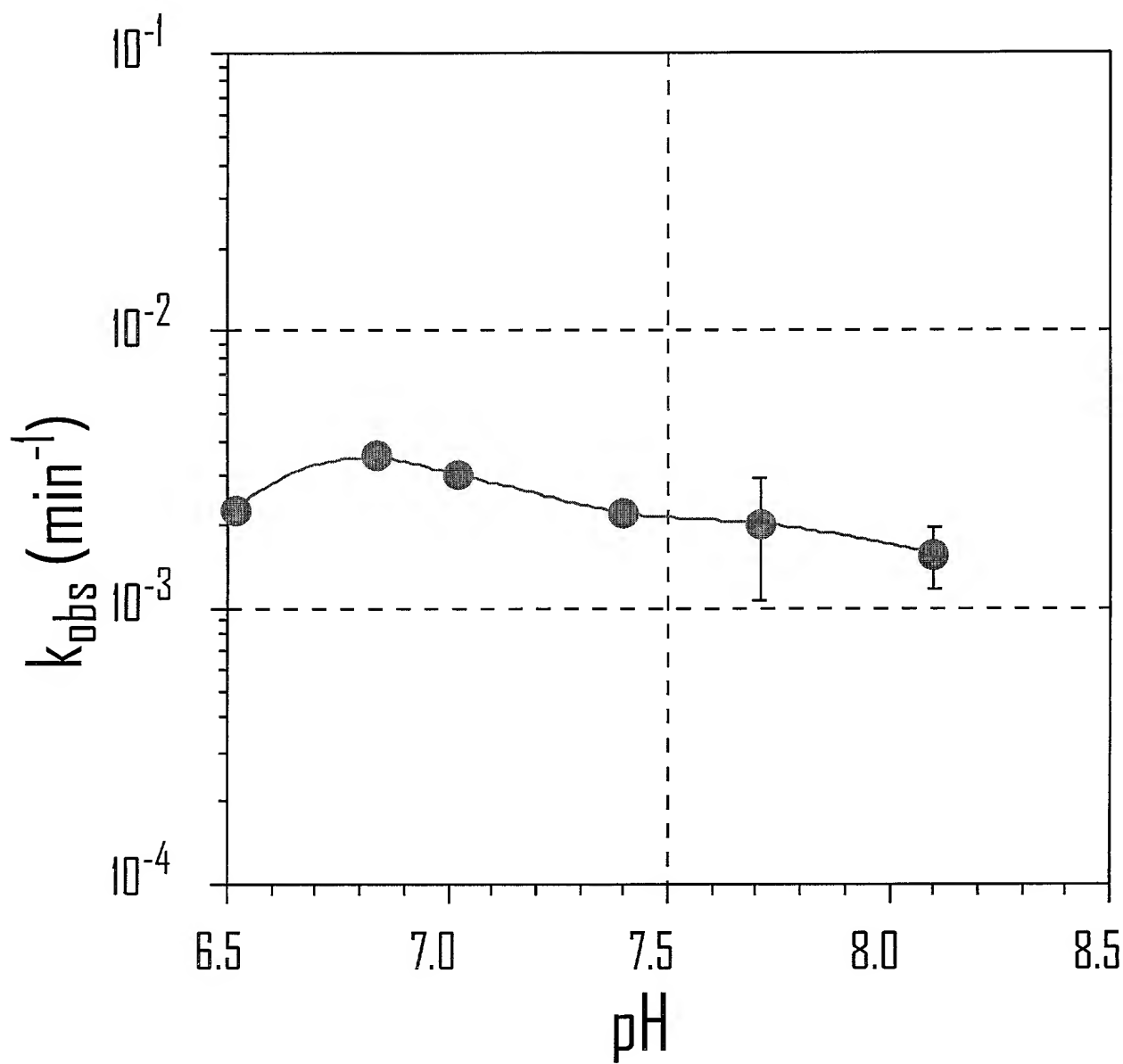
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Figure 37B



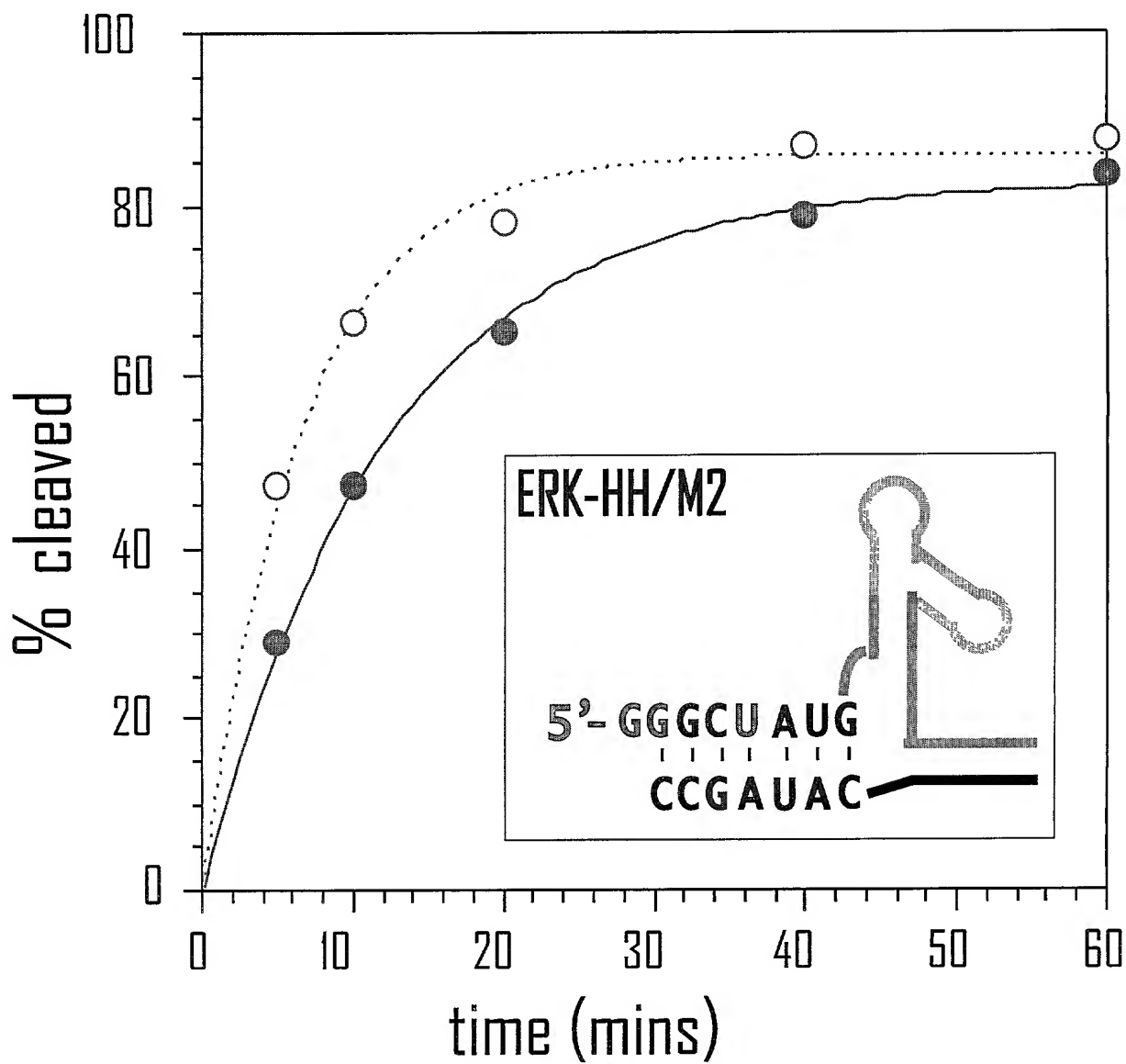
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Figure 37C



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Figure 37D



20250101 10:56:10

Figure 38

2022-10-19 15:00:07

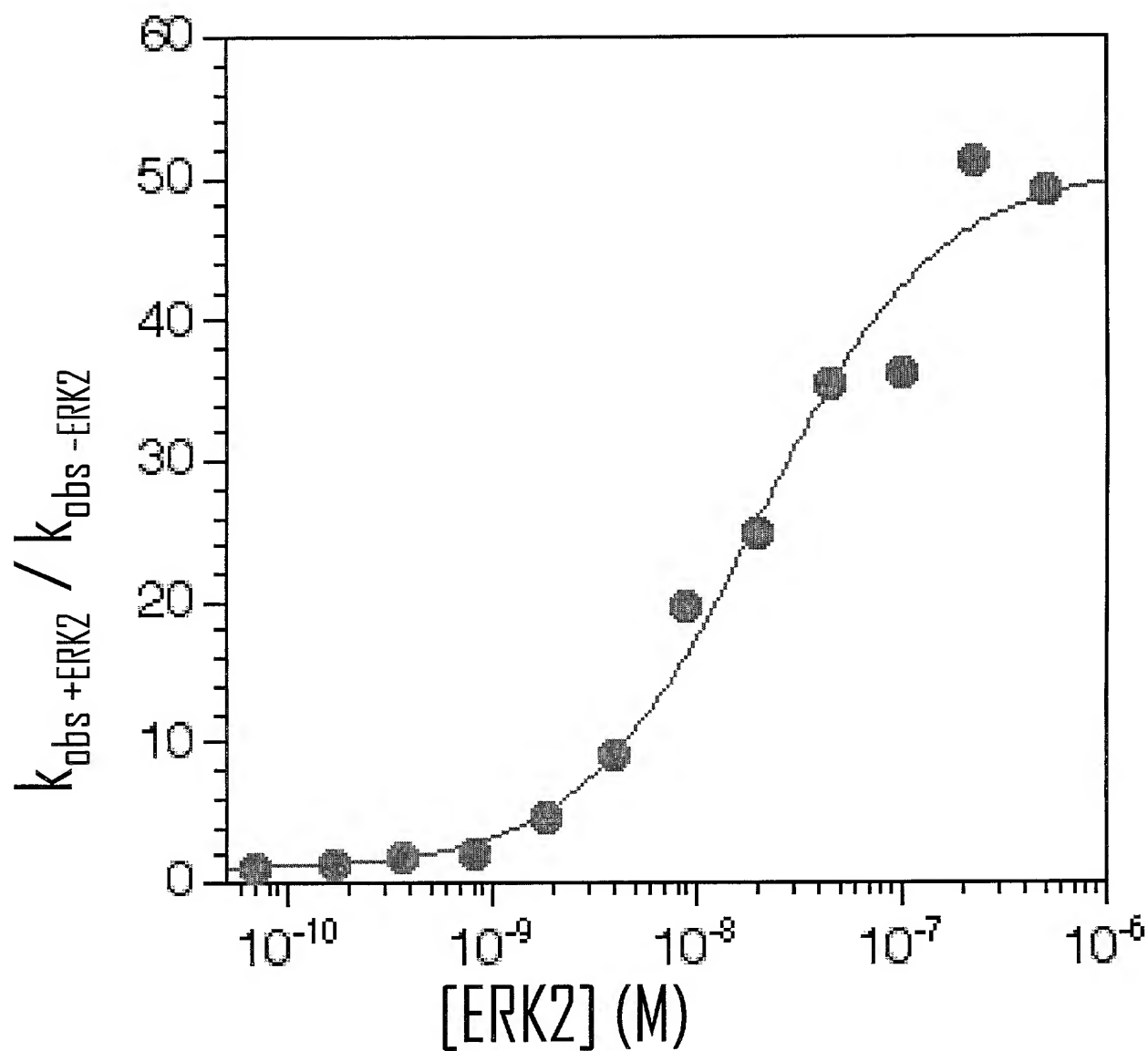
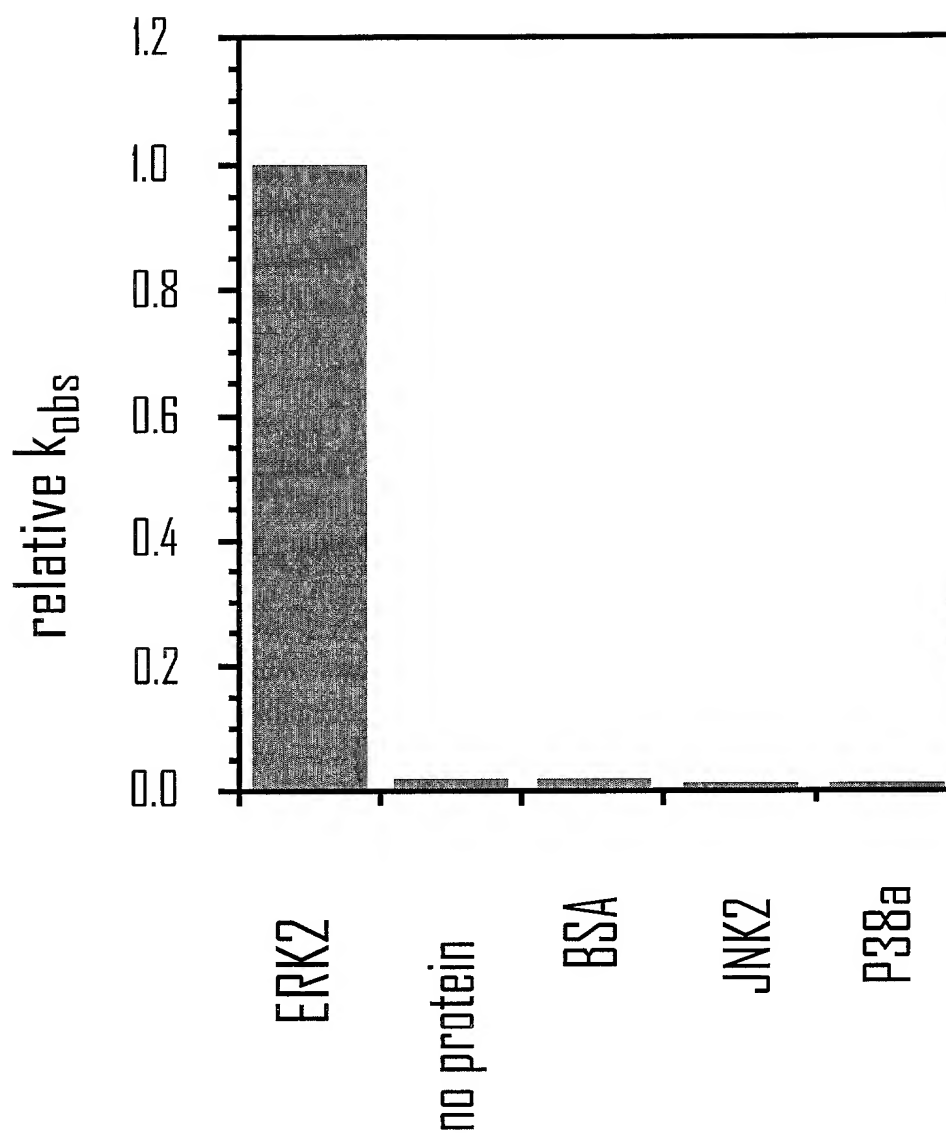
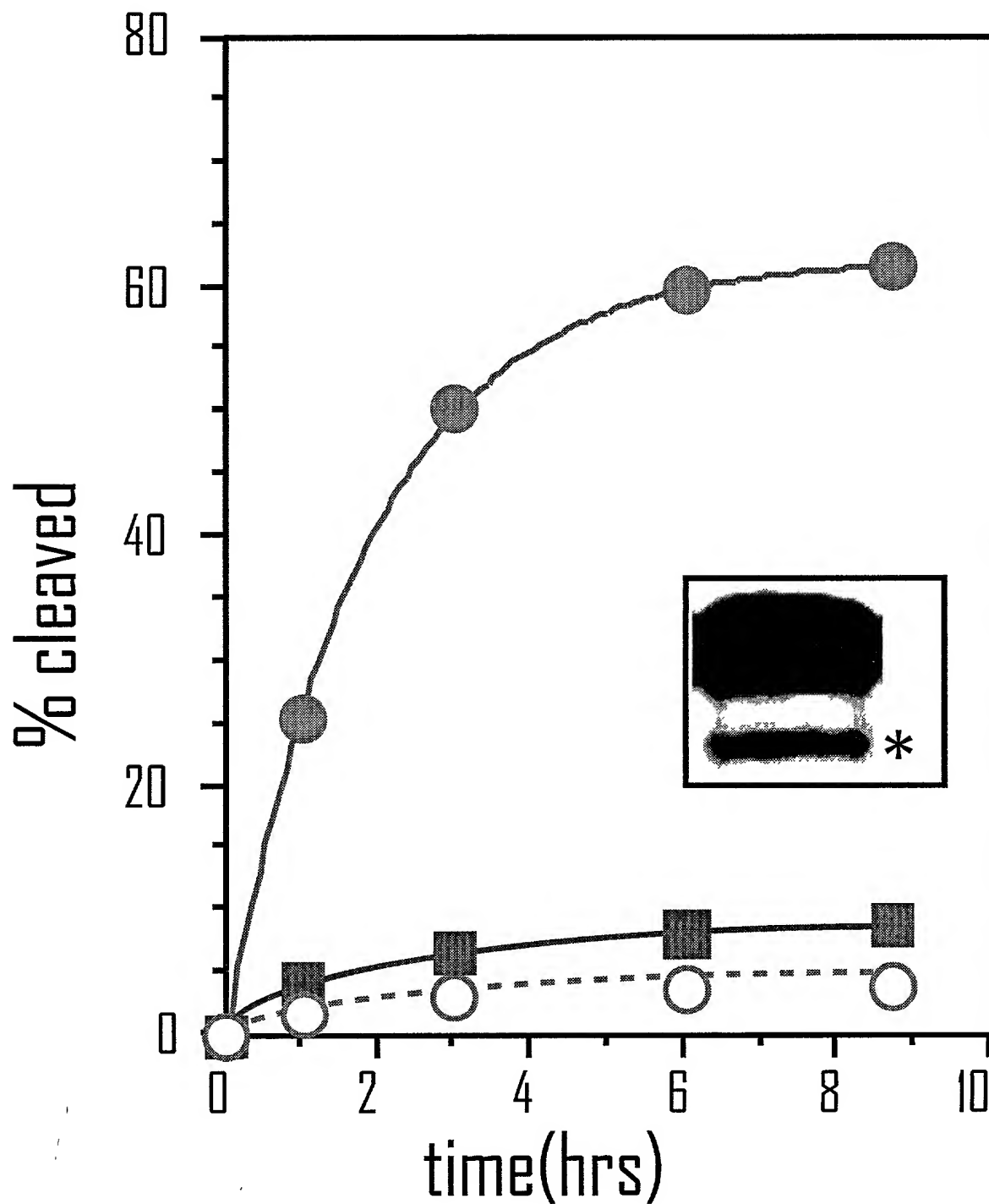


Figure 39A



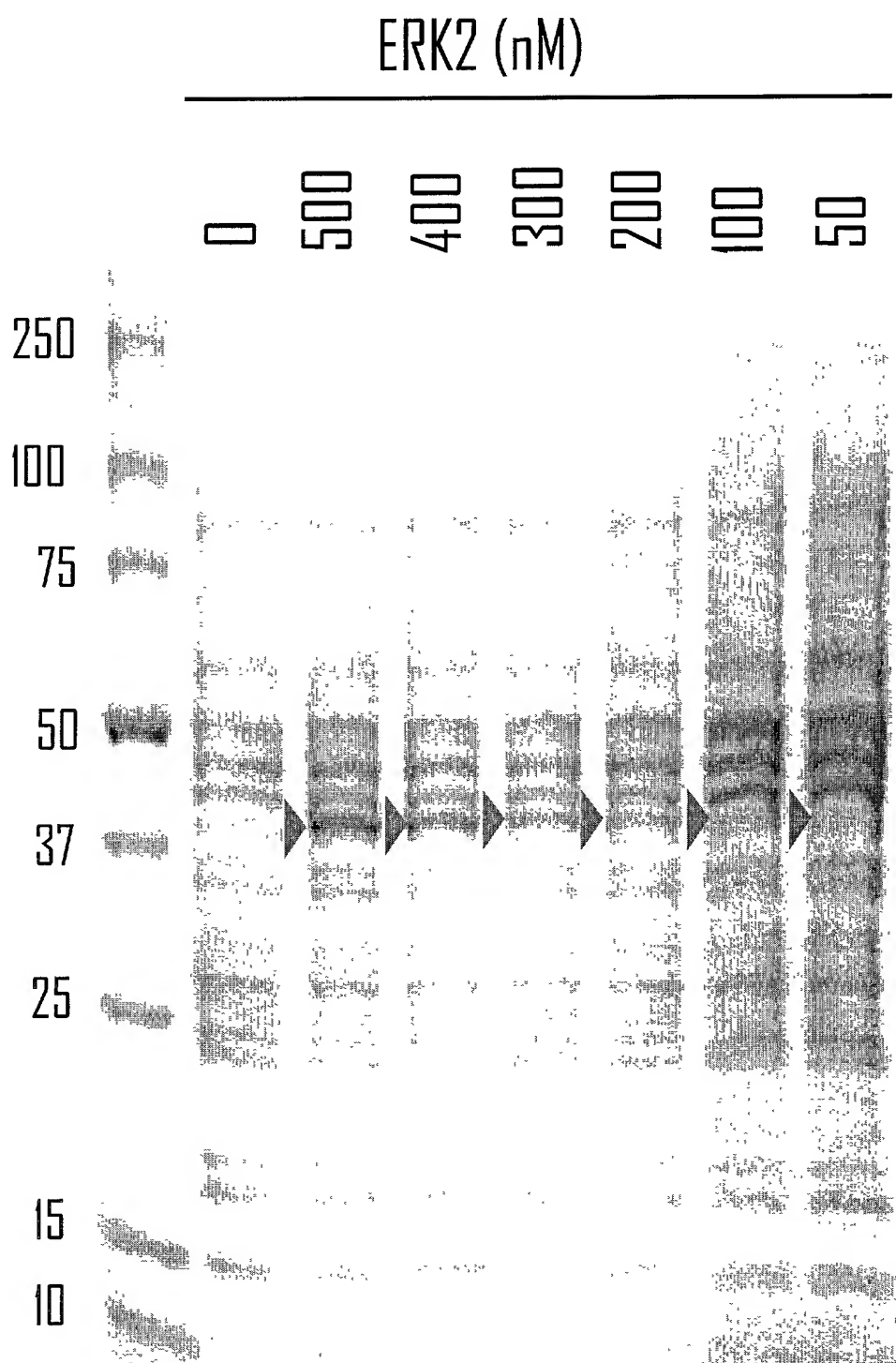
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Figure 39B



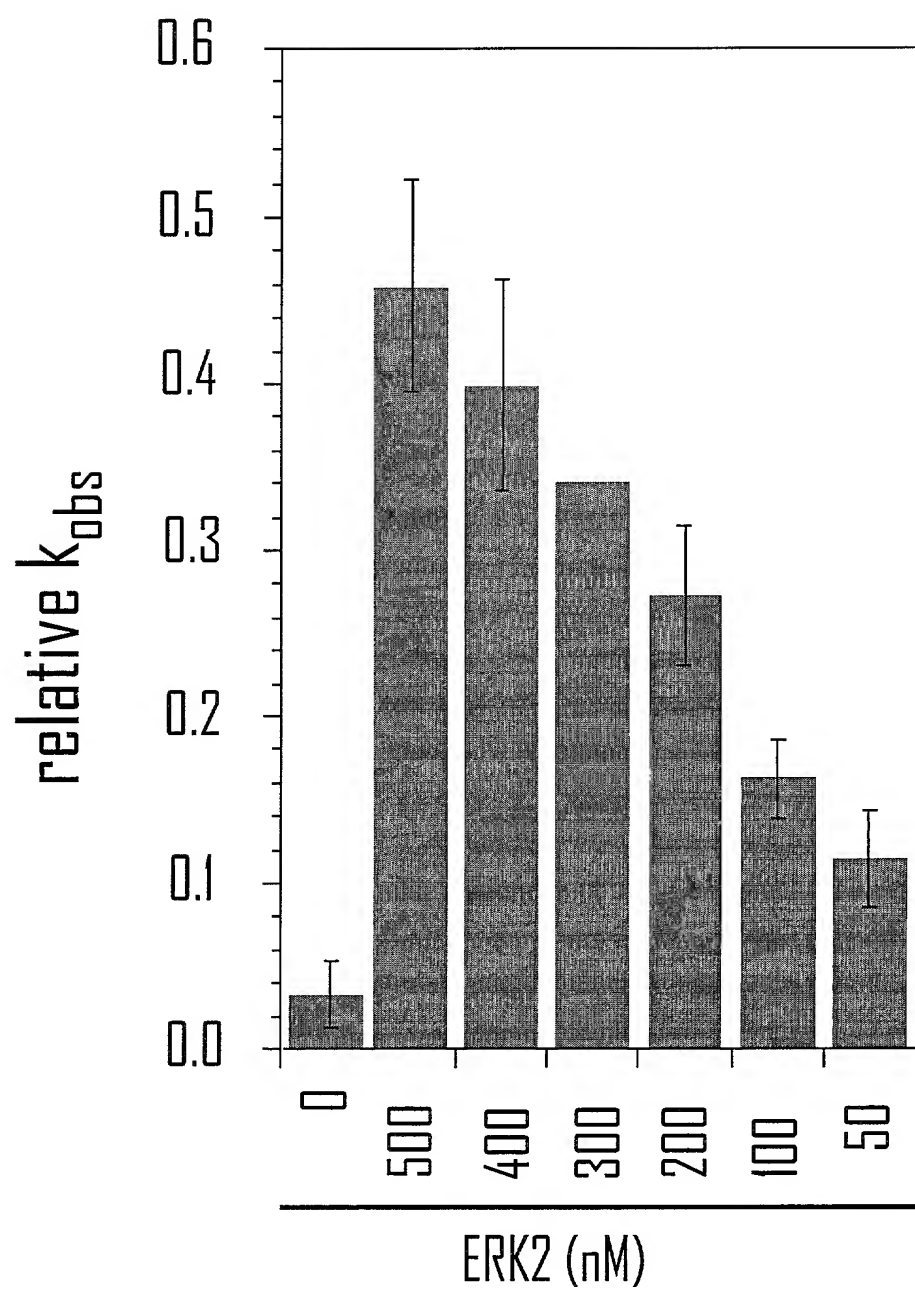
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Figure 40A



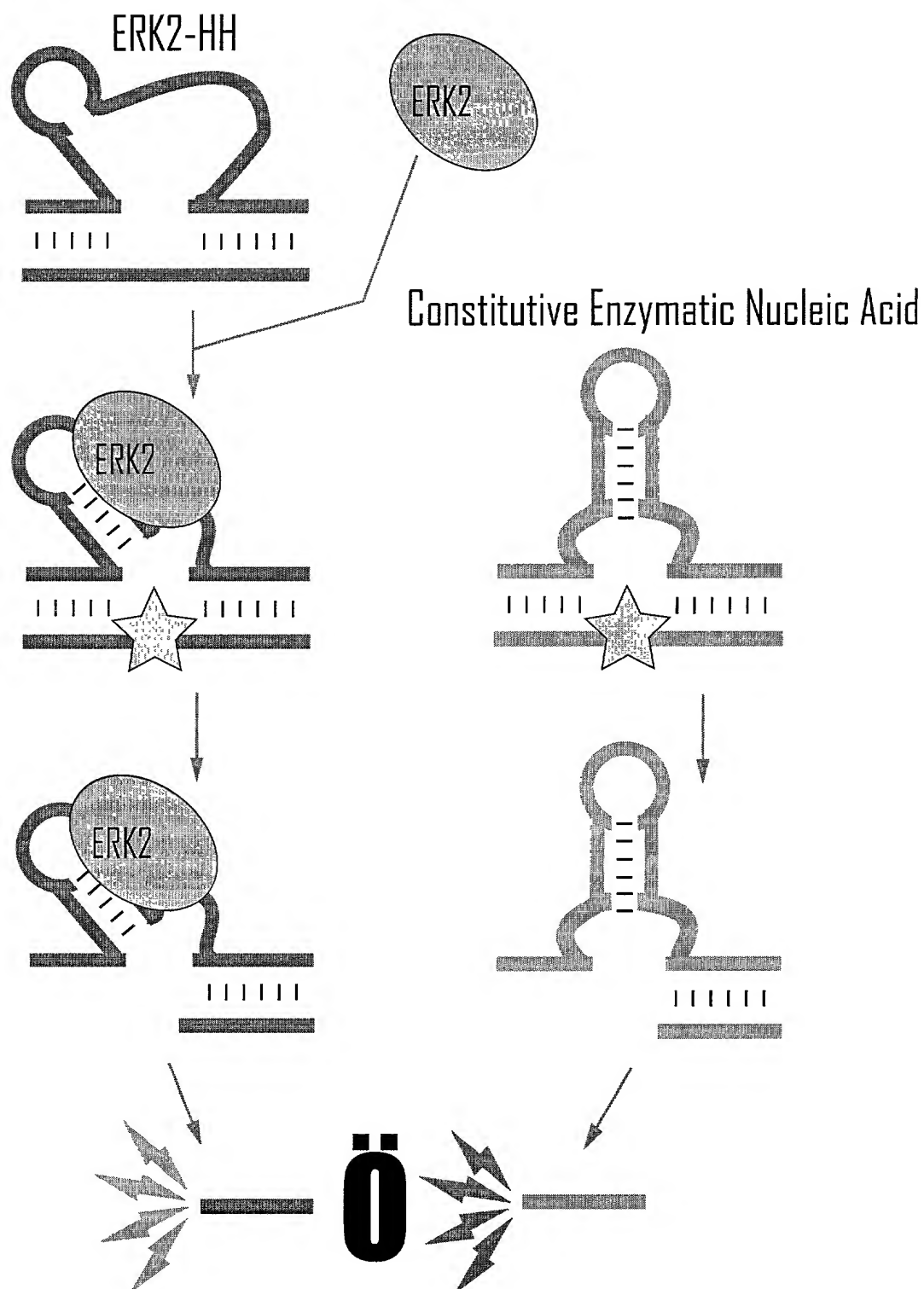
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Figure 40B



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Figure 41A



10056761.012302

Figure 41B

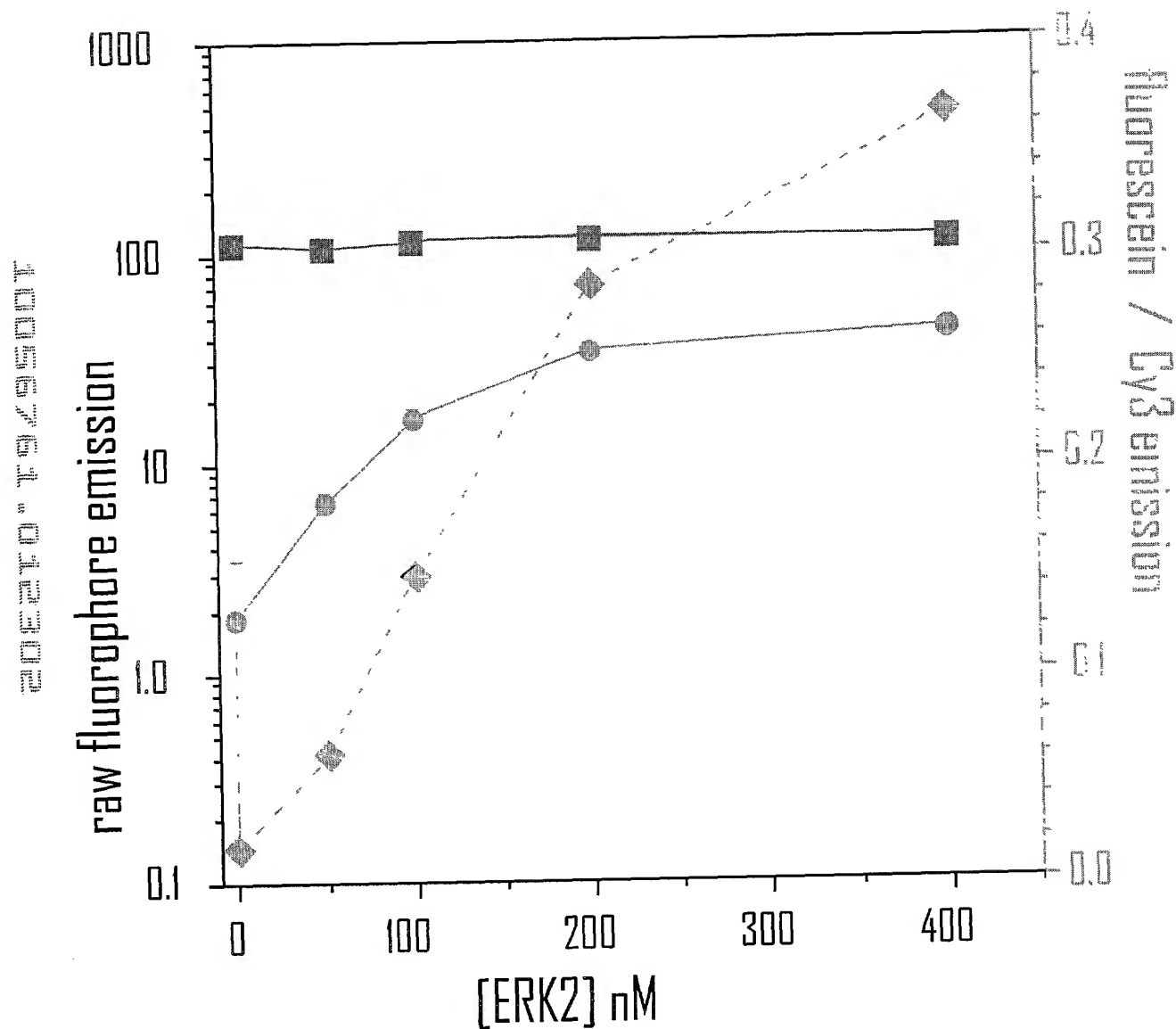
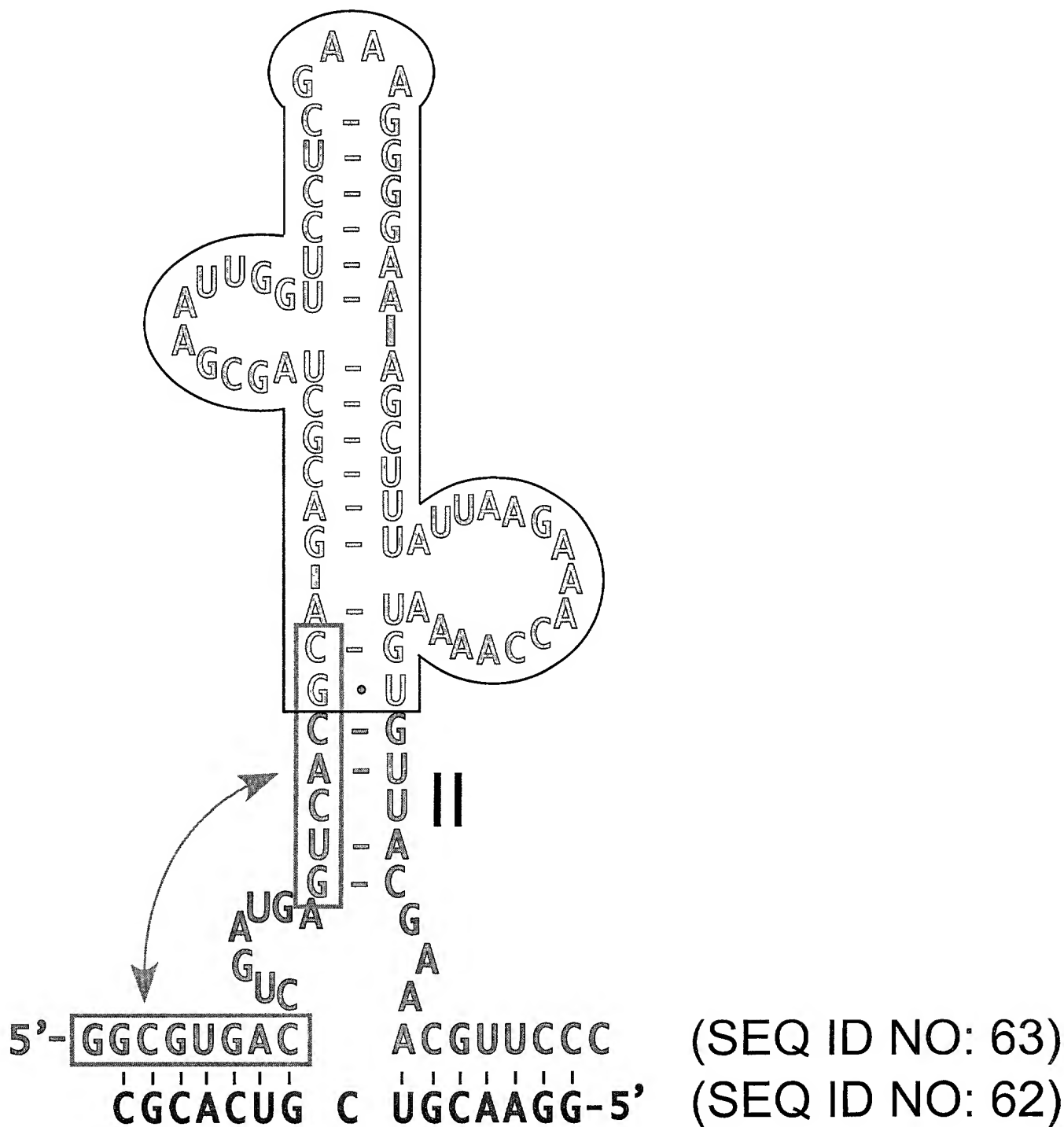


Figure 42A



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Figure 42B

